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A DISSERTATION FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

**Establishment of Theoretical Models for the Interpretation of
Nutrient Variations in Electrical Conductivity-based Closed-loop
Soilless Culture Systems**

전기전도도 기반 순환식 수경재배 시스템의 양분 변동 해석을 위한
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Establishment of Theoretical Models for the Interpretation of Nutrient Variations in Electrical Conductivity-based Closed-loop Soilless Culture Systems

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ABSTRACT

In general, nutrient management technology in closed-loop soilless culture system has been constructed based on electrical conductivity (EC) measurement, which is proportional to the total equivalent concentration of ions. However, the EC-based system is unable to detect changes in the concentration of individual nutrients that change dynamically depending on the environment and a systematic technique for control of the nutrient variations in soilless culture has not been presented yet. Furthermore, the closed-loop system may also be accompanied by potential problems from biological contamination due to reuse of drainage. In order to replace closed-loop soilless culture system with open-loop soilless culture system, it is necessary to secure operational stability through technical systematization. The objectives of this study were to establish the theoretical models for interpretation of nutrient variations in EC-based closed-loop soilless

cultures, to derive nutrients control techniques from theoretical analysis, and experimentally evaluate the developed technologies for spreading the closed-loop soilless culture systems. Basically, colony formation units in greenhouses were investigated to systematically distinguish the biological contamination problem caused by application of closed-loop soilless culture system from biological contamination of the reused solution and contamination of whole cultivation system due to nutrient discharge into the greenhouse. Changes in nutrient concentrations and ratios in closed-loop soilless culture system also analyzed in order to identify the aspect of the nutrient variation in the system and to extend it to theoretical analysis. Firstly, greenhouses using closed-loop soilless culture were investigated to be more advantageous condition for restraining microbial proliferation inside the greenhouse due to active drainage collection. In basic study on the nutrient variation, certain tendencies in nutrient balance changes even under the variations of the nutrient concentrations were observed. To extend this to theoretical analysis, theoretical models of EC-based soilless culture system were constructed and the basic study on the nutrient balance changes was subsequently expanded to theoretical and experimental analyses. Through the theoretical analyses, it was confirmed that the nutrient balance converged on the target value by long-term feedback even under the random walk disturbance in nutrient uptake concentrations. In addition, an alternative nutrient replenishment method (AM) was proposed to solve the problem of EC variation in closed-loop soilless culture system. The alternative nutrient replenishment method was basically aimed at performing follow-up change of total nutrient absorption in the system. The effect of AM was theoretically compared with the conventional nutrient replenishment method

(CM) through simulation analysis. Furthermore, in order to confirm whether these effects are reproduced in the actual cultivation system, a demonstration experiment was conducted. As the simulation analysis, the error between the total nutrient input and total nutrient uptake was minimized, and accordingly the fertilizer usage was reduced compared to the nutrient supplement method. Moreover, regular nutrient input was performed compared to the CM and changes in nutrient concentrations were observed in the average steady state. Finally, the nutrient balance control technique which is derived from the theoretical analyses was demonstrated under the AM applied EC-based closed-loop soilless culture system. Sweet pepper cultivation experiments were conducted under experimental and commercial scale greenhouse conditions, respectively and compared with the results of the open-loop soilless culture system. The demonstration experiment showed no significant differences from the open-loop soilless culture in quality and productivity of sweet pepper and the convergence of nutrient changes within target values were observed in the closed-loop soilless culture. Based on the theoretical models constructed in this study, the nutrient variation of the EC-based closed-loop soilless culture system was analyzed. The nutrient balance control technique is derived based on the theoretical analyses and demonstrated by the cultivation experiments. This approach can expect further technical advancement and extension, and consequently, can contribute to the technical systematization of closed-loop soilless culture system.

Additional keywords: closed-loop soilless culture; Michaelis-Menten equation; nutrient control; nutrient use efficiency; nutrient uptake model; plant mineral nutrition

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GENERAL INTRODUCTION

A large amount of nutrients and water are injected into the agricultural production system every year to increase productivity (Lassaletta et al., 2014; Tilman et al., 2002; Zhang et al., 2015). In particular, most of the nutrients are supplied from the outside of the system through fertilizers at a level that exceeds natural supply rate of nutrients, but a considerable amount is lost to the outside of the system (Lassaletta et al., 2014). This resource flow structure is causing instability in outside of the agricultural system, and there is a need to improve the flow structures for securing agricultural sustainability (Desmidt et al., 2015; Foley et al., 2005; Ortiz-Reyes and Anex, 2018; Tilman et al., 2002; Withers et al., 2014).

An agricultural system based on soilless culture can control essential factors of plant nutrition while physically blocking outflow of resources to the outside (Jones, 2005; Raviv and Lieth, 2008). Therefore, the soilless culture is a production system that can contribute greatly to improvement of the resource use efficiency in the agricultural production system. However, the discharge of nutrients and water from the soilless culture system has been pointed out as an economic and environmental problem from the beginning to the present (Beerling et al., 2014; Noordwijk, 1990; Olympios, 1999; Raviv and Lieth, 2008).

This is basically a situation which derives from the trade-off relationship between the nutrient control in the soilless culture system and the blockage of nutrient and water discharge. Therefore, it requires a technological transition for replacing the conventional nutrient management technique which has open-loop structure of nutrients and water flow (Beerling et al., 2014; Noordwijk, 1990). In other words, this can be seen as a situation due

to the absence of appropriate technology required for nutrient management in closed-loop structure. In addition to nutrient management, techniques such as sterilization and filtration are required for reuse of discharged nutrients and water, but these are already commercially viable techniques (Raviv and Lieth, 2008). However, in the case of nutrients, multiple factors are included in the control target because of essential elements, and dynamic and seemingly complex changes of each nutrient are observed in the root zone (Heinen, 1997; Noordwijk, 1990).

This has been a limiting factor in the systematic approach and the development of appropriate technology in the related studies so far. Therefore, most of the studies have been carried out through relative comparison by controlled experiments or by attempting on-line measurement of each nutrient with ion sensors (Cho et al., 2018; Gieling et al., 2005).

In the case of the controlled experiment, there was no proper theoretical platform for nutrients variation in the closed-loop soilless culture system, the stability of the cultivation has been verified by changing the terminal factors of the system such as the irrigation systems (Bouchaaba et al., 2015; Incrocci et al., 2006; Zekki et al., 1996), composition of the nutrient solution (Gent and Short, 2012; Hao and Papadopoulos, 2002; Neocleous and Savvas, 2018, 2015), and reuse period (Ehret et al., 2005; Ko et al., 2013). In this case, however, there may be limitations in the compatibility of the results or technical systematization. The real-time measurement studies have also been performed for a long time, but it is not replacing the conventional open-loop method in terms of technical stability and completeness in the measurement of various essential ions. Until recently,

this kind of stagnant situation has continued in related researches, and it is not connected with practical technological transition.

The objectives of this study were to establish the theoretical models for interpretation of nutrient variations in EC-based closed-loop soilless cultures, to derive nutrients control techniques from theoretical analysis, and experimentally evaluate the developed technologies for spreading the closed-loop soilless culture systems.

LITERATURE REVIEW

Diminishing returns in agricultural production system

The use of fertilizer and plant growth in agriculture was scientifically linked by Liebig's law of minimum, and the reductive viewpoint established in this process led to the deduction of the soilless culture system, which performs a high degree of control over the essential factors in plant nutrition, so that traditional agriculture systems could achieve an expansion of the production platform (Hoagland and Arnon, 1950; Jones, 2005; Marschner and Marschner, 2012; Raviv and Lieth, 2008). The total amount of fertilizer used has steadily increased since this technical transition, and this brought significant improvement in agricultural productivity (Foley et al., 2005; Tilman et al., 2002, 2001). However, the effect of increased fertilizer input was decreased over a long period of time. Now, diminishing returns are observed in agricultural production systems, and this process has been significantly affected by the loss due to the outflow of supplied fertilizer from the agricultural field. (Lassaletta et al., 2014; Swaney et al., 2018; Tilman et al., 2002; Zhang et al., 2015). In addition to the decrease in the efficiency of the agricultural production system, the outflow of fertilizer poses a serious threat to the sustainability of agriculture due to eutrophication, deterioration of soil and water quality, and a shortage of fertilizer raw materials (Desmidt et al., 2015; Foley et al., 2005; Ortiz-Reyes and Anex, 2018; Tilman et al., 2002; Withers et al., 2014).

Closed-loop soilless culture system

In general, the sustainability of resource management can be ensured through the collection and reuse of the outflow, which requires resource flows of closed loop structures (Desmidt et al., 2015; Fiksel, 2003). However, in soil-based systems, direct connections for the flow of nutrients and water are difficult (Granstedt, 2000), whereas a soilless culture system can expect an ideal management of nutrients and water, which maximizes productivity through the precise control of nutrients and water and physically blocks resource outflow (Jones, 2005). These features mean that it can be suggested as a sustainable resource management model, which can contribute to improving resource-use efficiency in agriculture. However, paradoxically, soilless culture has been regarded as an effluent source of nutrients at high concentrations due to the open-loop supply practices of nutrients and water, which repeatedly produces a certain percentage of drainage and the intensive use of fertilizers (Beerling et al., 2014; Noordwijk, 1990; Olympios, 1999; Raviv and Lieth, 2008). As soilless culture is advantageous for precise control, root zone nutrients change more sensitively than soil cultivation. This structural limitation of the system itself requires frequent corrections for fluctuations in nutrients, and the problem of nutrients and water emission can be seen as a result of the conventional use of relatively simple nutrient management techniques that control the fluctuations in root zone by releasing a certain percentage of the drainage outside the system (Ku and Hershey, 1991; Noordwijk, 1990; Olympios, 1999).

Nutrient management techniques in closed-loop soilless culture system

A closed-loop soilless culture system that reuses discharged nutrients and water can minimize resource losses but is accompanied by nutrient variations resulting in nutrient imbalance and instability in crop production (Hao and Papadopoulos, 2002; Kläring, 2001; Raviv and Lieth, 2008; Zekki et al., 1996). Ideally, these variations can be minimized through the measurement of individual nutrients. Thus, the development studies for nutrient management systems using real-time measuring devices, such as ion sensors, have been conducted; however, as a primary nutrient control system replacing open-loop nutrient management techniques, there were technical constraints (Bratov et al., 2010; Gieling et al., 2005; Kläring, 2001; Lee et al., 2017). In general, nutrient management techniques in soilless culture systems have been constructed based on electrical conductivity (EC) measurements, which are proportional to the total equivalent concentration of ions and the technical approaches for nutrient management in closed-loop soilless culture that have been carried out mainly in an EC-based system, which is favorable for field applications and the dissemination of techniques (Jones, 2005; Moon et al., 2018; Raviv and Lieth, 2008). However, the EC-based system is unable to detect changes in the concentration of individual nutrients that change dynamically depending on the environment, and the nutrient variations have been a major resistance factor for the acceptance of the closed-loop soilless culture system (Beerling et al., 2014; Kläring, 2001; Lee et al., 2017; Raviv and Lieth, 2008). In the absence of adequate nutrient management technology to replace the open-loop system, there is a trade-off relationship between the improvement of crop productivity through the precise control of nutrients and water and the improvement of resource utilization efficiency through the prevention and reuse of

nutrients efflux. Furthermore, this situation is now making it difficult for each farm to regulate emissions and to switch to a closed-loop soilless culture system (Beerling et al., 2014).

Approaches to develop nutrient management techniques

The problems defined in the nutrient management of closed-loop soilless culture have been the changes in root zone nutrients resulting from fluctuations in nutrient uptake concentrations and the instabilities in crop production due to the reuse of the drainage reflecting these fluctuations (Kläring, 2001; Moon et al., 2018; Noordwijk, 1990; Zekki et al., 1996), and the approaches to problem solving under these constraints have been mainly focused on controlled experiments that are conducting an investigation and comparison between open-loop and closed-loop soilless culture. Thus far, various experimental results have been provided about the effects of nutrient changes on plant growth in closed-loop soilless culture systems; however, until now, these have not been linked to the techniques to replace the open-loop nutrient management. However, a top-down survey through the manipulation of parameters and variables in the system terminal by the controlled experiment may have limits to the interpretation of the system fluctuation that is fundamentally caused by the plants and the connection to the technical systematization through the technical and theoretical compatibility of previous studies. Therefore, the interpretations of the results of the previous studies on the stability of cultivation in closed-loop soilless culture system from various perspectives such as the nutrient-reuse period (Ehret et al., 2005; Ko et al., 2013), EC control (Rouphael et al., 2016; Signore et al., 2016),

semi-closed-loop systems (Massa et al., 2011; Rouphael et al., 2016), composition of nutrient solutions (Gent and Short, 2012; Hao and Papadopoulos, 2002; Neocleous and Savvas, 2018, 2015), and differences in irrigation systems (Bouchaaba et al., 2015; Incrocci et al., 2006; Zekki et al., 1996) were carried out, and these were difficult to interpret in an integrated way. To ensure the usefulness and ease of closed-loop nutrient management techniques and to replace open-loop systems, the complexity of techniques and instability must be minimized, and in terms of research and technology development, an integrated and compatible platform for theoretical interpretation should be established. For this, the cultivation system and plant nutrition should be reduced as key parameters and variables and interconnected; moreover, nutrient control technology needs to be derived through a theoretical basis.

Nutrient model and plant stoichiometry

Basically, the nutrient uptake phenomenon of a plant follows the Michaelis-Menten equation (Clarkson, 1985; Cott et al., 2018; Le Bot et al., 1998). In the case of a system that follows this mechanism, it can have a steady-state solution according to the conditions of input variables and parameters (Golicnik, 2011), which means that the change in nutrient concentration in the plant root zone can converge to a specific value after a certain period of time. Furthermore, from the stoichiometric point of view, the accumulation of nutrients is explained as a chain of reactions depending on the biochemical composition of plants, so that in the ratio between nutrients, convergent changes are observed compared to the variation of individual nutrients (Ågren, 2008, 2004; Ågren et al., 2012; Kerkhoff and

Enquist, 2006; Knecht and Göransson, 2004). These suggest that the plant has its own nutrient balance control ability and stability, and it means that there is a field that is confronted with the basic premise of studies on closed-loop nutrient management thus far. Moreover, it is necessary to theoretically investigate the variables of the cultivation system for technical systematization. Previous studies using nutrient models in soilless culture systems have been conducted with the aims of model construction and verification (Heinen, 1997), analysis of nutrient absorption and inhibition (Silberbush et al., 2005; Silberbush and Ben-Asher, 2001), prediction of nutrient uptake and concentration change (Pardossi et al., 2005), and estimation of the optimal leaching ratio (Noordwijk, 1990). However, there have been no attempts to analyze the nutrient management and control techniques in the closed-loop soilless culture system in consideration of the abovementioned plant nutritional aspects.

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CHAPTER 1. Investigation of Potential Disease Occurrence and Nutrient Balance Change in Open- and Closed-loop Soilless Cultures

CHAPTER 1-1. Field Survey of Colony Forming Unit (CFU) of Microorganisms in Greenhouses using Open- and Closed-loop Soilless Culture Systems

CHAPTER 1-2. Ion Balance and Individual Ionic Contributions to EC Reading by Renewal Interval of Nutrient Solutions in EC-based Closed-loop Soilless Cultures

CHAPTER 1-1

Field Survey of Colony Forming Unit (CFU) of Microorganisms in Greenhouses using Open- and Closed-loop Soilless Culture Systems

ABSTRACT

The objective of this study was to compare colony forming unit (CFU) of microorganisms in closed- and open-loop soilless culture systems for estimating potential disease occurrence. Samples were collected at four different positions in four commercial greenhouses with closed or open soilless culture system using rockwool or coir as substrate, respectively. The distance between sampling positions was 3 cm starting from the substrate and the surface area of each position was 25 cm². The CFU of fungi was significantly higher in the open system, while that of bacteria was not significantly different but showed relatively lower in the closed system. Samples collected at the plastic surface of the substrates where little environmental effects occurred from drainage showed lower CFU than any other positions. The principal component analysis showed that samples collected on the drainage pathway highly affected the changes in microbial population in the greenhouse. These results indicated that greenhouses applied with closed soilless culture are expected to be more advantageous condition for restraining microbial growth, indicating the lower potential of disease infection in greenhouse ecosystem.

Additional keywords: commercial greenhouse; disease; drainage; greenhouse;
environment

INTRODUCTION

In soilless culture systems using substrates, salinity accumulation in root-zone and nutrient-balance change may occur, and a certain proportion of drainage is required to correct the fluctuation (Schon and Compton, 1997). In this process, nutrients such as nitrate are leached at high concentrations, which act as sources of pollutant on groundwater (McAvoy, 1994). Therefore, it is necessary to increase the resource use efficiency and reduce the environmental pollution source by applying closed-loop soilless culture system (Van Os, 1999), but the instabilities in the system such as nutrient imbalance (Zekki et al., 1996) and accumulation of organic acids (Lee et al., 2006) are acting as a limiting factor. This is an unstable factor for the application of the closed-loop soilless culture system circulation hydroponic cultivation method of farmers, and it is a part that needs technical stabilization.

Since soilless culture has a root zone environment isolated from the outside and soil, it is possible to maintain the population and species of the microorganisms at a relatively low level compared to the soil cultivation. However, these pathogens in greenhouses can be propagated through water, soil, and air (Agrios, 2005). Therefore, the increase in microbial populations inside the greenhouse can cause pathways to spread the disease and increase the likelihood of transmission. In addition, environmental conditions such as temperature and humidity (Etchells et al., 1973) and nutritional conditions (Chang et al., 2007) may affect the growth of microbial populations.

In the case of the greenhouse with open-loop soilless culture system, there is no active collection of drainage compared to closed-loop soilless culture system, and some are left in the greenhouse or released to the outside. Especially in most greenhouses with open-loop soilless culture, drainage during the irrigation is released inside the greenhouse and evaporates. Structural differences between open and closed-loop soilless culture system can cause changes in micro-environmental conditions and nutrient conditions in the greenhouse, which can affect the growth of pathogens. Therefore, the application of the closed-loop soilless culture system would provide a favorable condition for inhibiting the disease occurrence due to the closed circulation structure of the drainage in the greenhouse compared with the open-loop soilless culture system. If we can confirm the difference in the number of microbial populations according to the hydroponic cultivation system, we can consider the improvement effect of the sanitary condition in the greenhouse along with the resource saving and the environmental load reduction. The objective of this study was to investigate the variation of microbial population in the greenhouse by collecting drainage from closed-loop soilless culture system and to analyze the level of CFU of the potential pathogen in commercial farms adopting open or closed-loop soilless culture system.

MATERIALS AND METHODS

Conditions of investigated greenhouse

This experiment was carried out to investigate commercial greenhouse of glasshouse with a complex environmental control system of 1ha or more growing sweet pepper (Table 1.1.1). Two farms which are using coir and rockwool, respectively, were selected from each farms using open- and closed-loop soilless culture system, respectively, and samples were collected from October 24 to 26, 2012. All the farms were controlled by the solar radiation integral drip irrigation system, and the nutrient solution was controlled for 30% of drainage. The drainage of the closed-loop soilless culture system was sterilized by UV sterilization system and the generated drainage was stored in the primary catchment tank through the drainage line of the hanging gutter (Fig. 1.1.1A). The drainage from open-loop soilless culture farms was transferred to a drainage tank in the greenhouse through a styrofoam bed located between the two rows of the growing substrate, or stagnated in the greenhouse (Fig. 1.1.1B). The cultivation floor was covered with a covering material, and all were sprayed with a disinfectant about two weeks ago.

Sampling and culture of microorganisms

Samples were collected from the substrate cover, gutters, and greenhouse floor. The angle of a sterile disposable swab was maintained at about 30 ° when collecting samples from the surface (3M quick swab, 3M, USA). The swabs used in the collection were sealed in the collected liquid, and they were refrigerated and transferred to the ice box. The samples were collected at 4 points at a distance of 3 cm from a square area of 25 cm² on

the basis of the cover material of the substrate (Fig. 1.1.1), and the collection work was repeated three times at random in each greenhouse. Samples were diluted with sterile distilled water at a magnification of 10, 100 and 1000 times. The medium containing 30-300 colonies of dilution was used for counting the number of colonies. When more than 300 colonies existed at the maximum dilution ratio, the average number of colonies per unit area was calculated and then the number of colonies was calculated by multiplying the culture area. The cultures were incubated with 3M dry film media, 3M Petrifilm aerobic count plate (3M, USA) and 3M Petrifilm yeast and mold count plates (3M, USA). General bacteria were cultured at 35 °C for 48 hours, and fungi were cultured at 25 °C for 72 hours. Colony forming units (CFU) per unit area after culturing were calculated as follows.

$$\frac{CFU}{cm^2} = \frac{\text{Number of colonies on film} \times \text{Dilution ratio}}{25cm^2} \quad Eq. 1-1$$

All population data were converted to log colony forming units per cm². A comparison of the mean values of the microbial CFUs was made by means of variance analysis and principal component analysis was used to assess the impact of the sampling points on the level of microbial populations. For statistical analysis, SAS 9.3 (SAS Institute, Cary, NC, USA) was used.

Table 1.1.1. Soilless culture systems for the field survey.

Greenhouse type	Soilless culture system	Substrate	Drainage transport
Glasshouse, > 4 ha	Closed ^z	Rockwool	Hanging gutter
Glasshouse, > 4 ha	Closed	Coir	Hanging gutter
Glasshouse, > 2 ha	Open	Rockwool	Styrofoam bed
Glasshouse, > 4 ha	Open	Coir	Styrofoam bed

^zClosed and open mean closed and open soilless culture systems, respectively.

RESULTS AND DISCUSSION

The CFU values of the closed-loop type farms were significantly lower than those of the open-loop type farms (Table 1.1.2). The CFU values of the fungi were significantly lower than those of the open-loop type farms. Although there was no significant difference in bacterial counts, there was a trend of 3.65 log CFU / cm² in the greenhouse with closed-loop soilless culture system using coir substrate, 3.78 log CFU / cm² in the same substrate condition, The CFU of the greenhouse was 2.66 log CFU / cm², which was lower than that of 3.18 log CFU / cm² in the greenhouse with open-loop soilless culture system. The increase in the number of microorganisms in the greenhouse can be influenced by the difference in the environmental conditions depending on the collection method of the drainage and may be influenced by the composition of the drainage which can act as a sort of medium for the microorganisms have. The greenhouses included in this study used rockwool and coir as the substrate, respectively, but there may be differences in the components of the drainage as inorganic and organic media. As a result of CFU analysis in the greenhouse, the CFU values of the fungi were significantly lower in the circulating hydroponic greenhouse using the rockwool. However, the greenhouse with closed-loop soilless culture using coir as a substrate was similar to that of the open-loop soilless culture system using rockwool. The highest CFU value in fungi appeared as the open-loop soilless culture system using coir as a substrate. High values in fungi were observed in closed-loop and open-loop soilless culture system where coir substrate were used. The coir medium consists of coconut fiber, which can drain organic matter (Domeño et al., 2009). Differences in the content of organic matter can also affect microbial activity and

population growth (Chang et al., 2007). The above results for the substrate are judged to be interference due to the qualitative difference in drainage. The non - recirculating hydroponic greenhouses are relatively inferior in the minimization of drainage exposure to the closed-loop soilless culture system in that the drainage pathway is relatively narrow, which minimizes the increase in the number of microbial populations. These results suggest that the effects of factors such as the disinfectant spraying of the farmers as well as the effects of open-loop and closed-loop drainage collection facilities, can't be excluded. However, Rowe and Farely (1978) reported that re-colonization occurred within 2-3 weeks after microbial input into sterilized soil in tomato growing greenhouses. All of the farms surveyed were sprayed with fungicide about two weeks ago. Therefore, the interference effect on the survey result is considered to be minimized.

Sample collection areas for both open and closed-loop soilless culture system are shown in Fig. 1. In case of the P1 and P2 areas, they are functionally similar as the substrate and drainage collection area in both of closed- and open-loop soilless culture system. However, in case of the P3 and P4 areas, these consisted of drainage collection part in open-loop system and of greenhouse floor in closed-loop system (Figure 1.1.1B). Open-loop soilless culture does not require active collection of drainage, so some are released to the outside of the greenhouse or become stagnant near the substrate. As a result, the surface of the P3 and P4 zones (Fig. 1.1.1A) of the closed-loop soilless culture system provides different levels of environment in the growth of microorganisms compared to the same zone of the open-loop soilless culture. Since the growth rate of microbial populations can be determined by the initial colony number, environmental conditions such as temperature

and humidity, and inorganic nutrients (Etchells et al., 1973; van Maanen and Xu, 2003; Sridhar and Bärlocher, 2000), the differences in the number of microbial populations due to differences in the structure of soilless culture of the surveyed farms were judged to be the direct or indirect influence of the environmental conditions on the sampling surface to the microbial propagation.

The results of comparing the CFU values of the samples collected in the greenhouse are shown in Fig. 1.1.2. In the case of fungi in the open-loop soilless culture, the P2 - P4 zone showed the highest level, while the P1 zone was found to be the lowest with the same zone of the recirculating greenhouse. In the case of bacteria, no significant difference was observed for each zone, but CFU of the P1 zone of the open- and closed-loop soilless culture showed the lowest trend. The P1 zone had similar environmental conditions as both the open- and closed-loop soilless culture as covering areas of the substrate. This can be regarded as a difference because it was exposed to different environmental conditions at different locations even in the open-loop soilless culture system. The CFU of the fungi in closed-loop soilless culture was the lowest in all areas compared to the open-loop soilless culture. The CFU of the fungi was lower than that of the open-loop soilless culture system. Therefore, it can be inferred that the results of this study showed that the difference in environmental conditions due to the application of the open- or closed-loop soilless culture system rather than the interference of other factors had a large effect on the increase of microbial populations.

As a result of principal component analysis in which the sampling points of the samples in each greenhouse were used as the explanatory variables and the microbial

population level was set as the objective variable. The major component of the open-loop soilless culture using the coir in the first main component was the highest in the fungi. Thereafter, the open-loop soilless culture system using the rockwool, the closed-loop soilless culture using the coir, and the closed-loop soilless culture using the rockwool were shown (Fig 1.1.3). The results of the first principal component contributed the largest contribution to the P2 area, but the P3 and P4 areas showed similar levels and the P1 area was the lowest. In the case of bacteria, open-loop soilless culture showed high level of main component score, but closed-loop soilless culture using coir showed similar score (Fig. 1.1.4) However, even in the case of bacteria, the closed-loop soilless culture using the rockwool showed a low level of main component scores. The P4 zone contributed the largest contribution to the principal component analysis of the fungi, and the P3 zone contributed to the same level. In the case of bacteria, the P1 zone was also found to contribute to the lowest level. This means that the P2 - P4 area on the drainage pathway which acted as a difference in the environment in the greenhouse is a variable that influences the change of microbial population in the greenhouse. This means that the influence of the P1 zone, which is less affected by the environment.

The three components that make up the onset of disease can consist of pathogen, environment, host plants that can be expressed as total sum of pathogenic strength and density, and the interrelationship between these elements can be expressed as a pathogen triangle (Agrios, 2005). In the case of a greenhouse, it is composed of a single crop and has a condition that can favor the occurrence of disease in terms of the number of host plants. In the case of an open-loop soilless culture system, it is considered that the

environmental and pathogen elements are higher than the closed-loop soilless culture system. In this study, only the total population of microorganisms was examined, so it is not known whether the microorganisms constituting this group are pathogenic or non-pathogenic. However, when the number of specific plants increases in a single space, the number of pathogens tends to increase together (Flory and Clay, 2013). The greenhouse using the open-loop soilless culture system satisfies the three elements of the bottle triangle at a relatively higher level, and therefore, in terms of potential illness occurrence, the open-loop soilless culture system has a higher possibility than the closed-loop soilless culture system. Although closed-loop soilless culture system is concerned with the accumulation of pathogenic microorganisms in drainage due to the reuse of drainage, the open-loop soilless culture is not suitable for the change of the microenvironment in the greenhouse due to the low technical need for collection of drainage. It is necessary to consider the possibility of potential disease outbreaks. Due to the characteristics of this study, the number of domestic recirculating hydroponic greenhouses using solid substrate to be surveyed is extremely small. However, as a whole, it is considered that the increase of microbial populations in the greenhouse due to the internal discharge from open-loop soilless culture. Therefore, application of appropriate drainage sterilization technology and drainage collection system for the closed-loop soilless culture systems are expected to provide relatively favorable greenhouse conditions in terms of potential disease prevention.

Table 1.1.2. Colony forming units (CFU) of fungi and bacteria according to soilless culture system and growing medium.

Growing medium	Soilless culture system	Colony forming unit (Log CFU/cm ²)	
		Fungi	Bacteria
Rockwool	Open ^z	2.53b ^y	3.18ab
	Closed	1.03c	2.66b
Cair	Open	4.02a	3.78a
	Closed	2.63b	3.65a
Significance			
	Growing medium (A)	***	*
	Soilless culture system (B)	***	NS
	A×B	NS	NS

^zOpen and closed mean open- and closed-loop soilless culture systems, respectively.

^yMean separation within columns by Duncan's multiple range test at $P=0.05$.

NS,*,*** Not significant or significant at $P=0.05$ or 0.001, respectively.

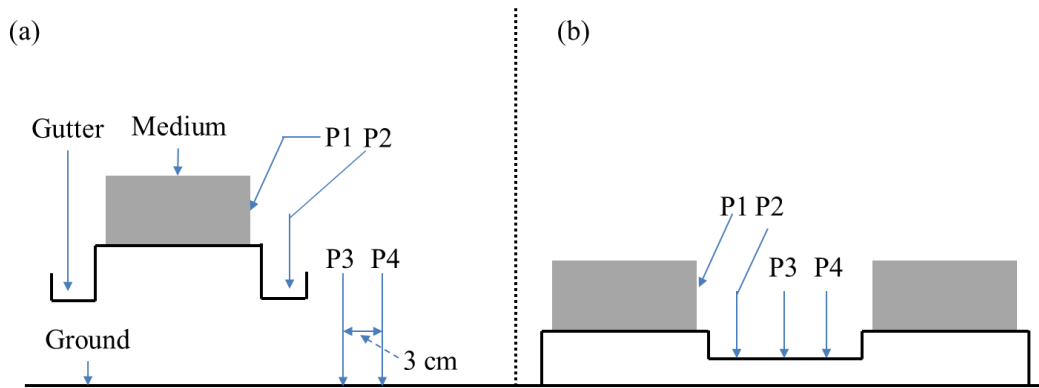


Fig. 1.1.1. Positions for microbial sample collection in the closed (A)- and open (B)-
loop soilless culture systems.

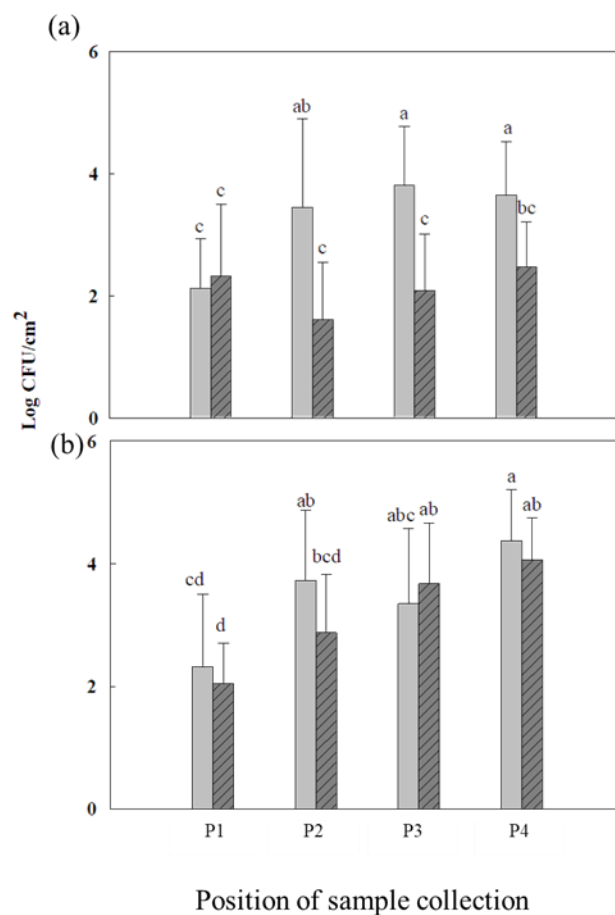


Fig. 1.1.2. Colony forming units (CFUs) of fungi (A) and bacteria (B) according to sampling position in the open (■)- and closed (▨)-loop soilless culture systems. Duncan's multiple range test at $P=0.05$. Refer to Fig 1 for the positions of P1, P2, P3, and P4.

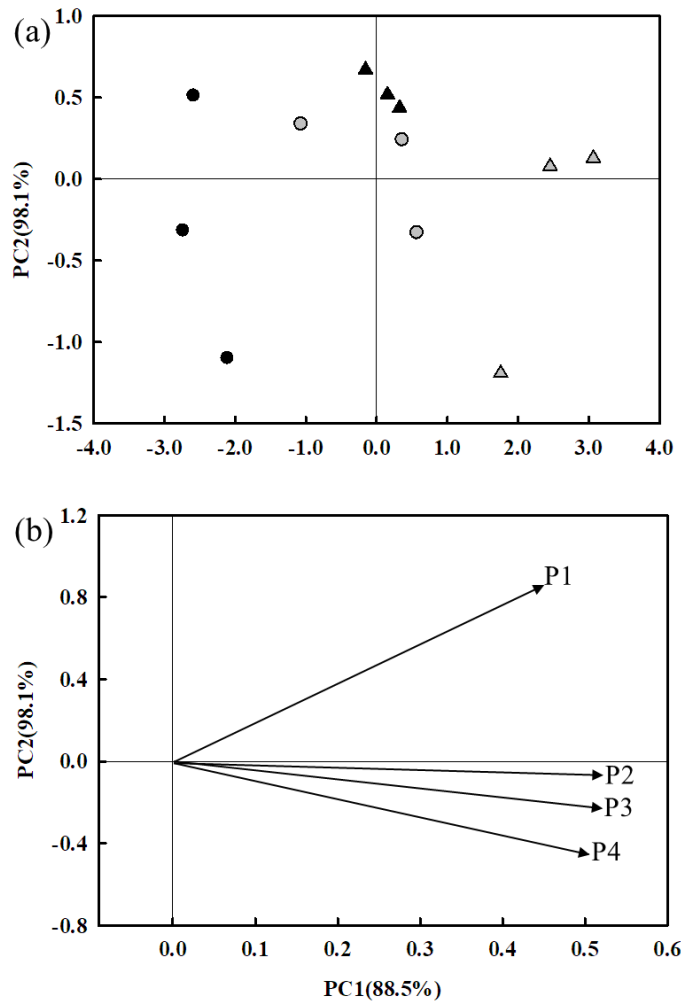


Fig. 1.1.3. Principal component analysis between CFU and sampling position in the greenhouse for fungi: PCA scores (A) of closed-coir (▲), closed-rockwool (●), open-coir (△), open-rockwool (○), and eigenvector plot (B). Closed and open mean closed- and open-loop soilless culture systems, respectively. Refer to Fig 1 for the positions of P1, P2, P3, and P4.

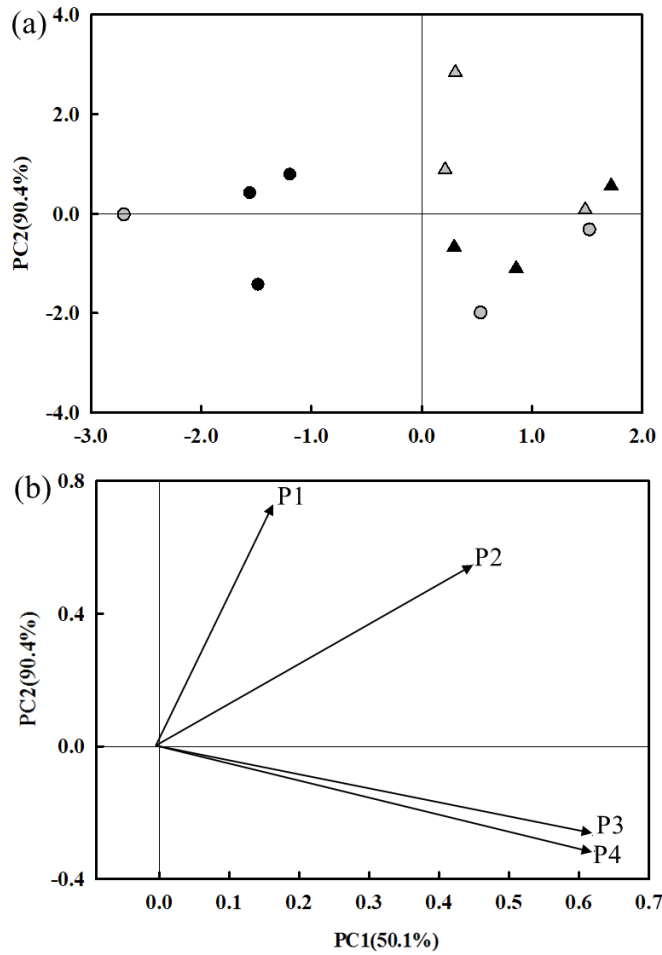


Fig. 1.1.4. Principal component analysis between CFU and sampling position in the green house for bacteria: PCA scores (A) of closed-coir (▲), closed-rockwool (●), open-coir (△), open-rockwool (○), and eigenvector plot (B). Closed and open mean closed and open soilless culture systems, respectively. Refer to Fig 1 for the positions of P1, P2, P3, and P4.

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CHAPTER 1-2

Ion Balance and Individual Ionic Contributions to EC Reading by Renewal Interval of Nutrient Solutions in EC-based Closed-loop Soilless Cultures

ABSTRACT

Individual ion concentrations and ionic contributions to EC reading in the circulated nutrient solution are important factors to be considered for stable EC-based closed-loop soilless culture. The objective of this study was to determine appropriate ion-analysis intervals of the circulated nutrient solutions based on ion concentration, ion balance, and ion electrical conductivity under different renewal intervals in EC-based nutrient control systems for sweet peppers (*Capsicum annuum* L. 'Fiesta'). Average node numbers of the plants were 13 and 18 when the experiment started and finished, respectively, and three plants were grown in each rockwool slab. Four different renewal intervals of circulated nutrient solutions such as 1, 2, 3, and 4 weeks were used as treatment. Nutrient solutions were supplied to the plants based on integrated radiation. Drainage was collected into drain tanks after irrigation ended in the day and then mixed with fresh water until the EC reaches $2.69 \text{ dS} \cdot \text{m}^{-1}$. The replenished nutrient solution was supplied to the plants in the next day. Ion concentrations of the individual ions periodically analyzed in the circulated nutrient

solutions showed no significant differences among the treatments during the experimental period. Ion concentrations of K^+ , Ca^{2+} , Mg^{2+} , Na^+ , NO_3^- , SO_4^{2-} , PO_4^{3-} , and Cl^- varied within 5 - 8, 11 - 14, 2.0 - 2.7, 0.5 - 0.6, 14 - 19, 4 - 5, 1 - 4, and 0.3 - 0.5, respectively. Ion balance showed a consistent tendency over all the treatments and especially $K^+ : Ca^{2+}$ and $SO_4^{2-} : PO_4^{3-}$ played great roles in the cation and anion balances in the nutrient solutions, respectively. Activity coefficients of ions such as K^+ , NO_3^- , and $H_2PO_4^-$ varied within 0.8 - 0.9 and those of Ca^{2+} , Mg^{2+} , SO_4^{2-} varied within 0.5 - 0.6, showing little changes with time. Ionic contributions of K^+ and NO_3^- to EC reading were the greatest followed by Ca^{2+} , SO_4^{2-} , and Mg^{2+} in the order. From the results, we thought that allowable ranges in ion concentration, ion balance, and subsequent individual ionic contributions to EC reading would be obtained within 4-week renewal interval of nutrient solution in EC-based closed-loop soilless culture for sweet pepper plants.

Additional Key words: activity coefficient; equivalent ionic conductivity; ion concentration; rockwool

INTRODUCTION

In order to prevent the accumulation of salts in the medium in soilless cultures, an excess amount of the nutrient solution is irrigated more than required to generate a certain amount of drainage. Drainage can be an environmental load when discharging because of the high concentration of salt. Therefore, it is necessary to apply the closed-loop soilless culture system. However, since the generated drainage is different in ion concentration and ratio from the irrigated nutrient solution, the drainage reuse without adjustment of ion concentration could be an unfavorable condition in plant nutrition. Currently, there is no established technology for stable reuse of drainage, so researches are needed to establish an eco-friendly cultivation system.

In order to manage the nutrient solution of closed-loop soilless culture system, studies have been attempted to directly measure the ion concentration of drainage and reuse as a nutrient solution (Gieling et al., 2005; Gutierrez et al., 2008), but due to technical limitations, it has yet to be commercialized. Therefore, instead of measuring the individual ions in the drain, measurement of the electrical conductivity (EC), which is the apparent concentration of the whole ions, is practically introduced to reuse the drain with a relatively high concentration. Savvas and Gizas (2002) studied a system for treating drainage based on plant nutrient uptake in EC-based closed-loop soilless culture system. The effects on growth effect were analyzed by changing the proportion of the cation in the nutrient solution used for calibrating drainage in closed-loop soilless culture of gerbera. In EC-based closed-loop soilless culture system, the treatment method of drainage can be effective at the time of nutritional imbalance. However, because ionic concentration and

ion balance in nutrient solution can't be corrected, applying EC-based soilless culture for long-term cultivation s can have a negative impact on plants (Ehret et al., 2005; Zekki et al., 1996). However, there is a case in which certain recirculation period does not have a negative effect on plant growth (Ehret et al., 2005; Raviv et al., 1998). But it does not provide clear results. In addition, the rate at which each ion concentration contributes to the EC in the nutrient solution also changes, so it is necessary to analyze this change pattern in the EC-based nutrient control state (Son, 1998).

Therefore, it is necessary to analyze the effect of each ion contributing to the EC, which is a control criterion, over a period during which the recycled nutrient solution can be used continuously and under these conditions for the stable drainage use. The objective of this study was to estimate the stable nutrient analysis period in closed-loop soilless culture by assuming the replacement cycle as analysis period of the recycled nutrient solution in the EC-based sweet pepper closed-loop soilless culture system and by investigating changes in nutrient concentration and EC-contribution.

MATERIALS AND METHODS

Plant cultivation and environmental condition

In September 2010, sweet pepper (*Capsicum annuum* L. cv 'Fiesta') was planted in an experimental glasshouse of Seoul National University at the time the first flower bud was formed and then cultivated in soilless culture system without drainage recycling. The nutrient recycling treatments were applied when the sweet pepper reached about 13 nodes. The average number of nodes at the end of the treatment was about 18 nodes. The average cumulative light intensity during the treatment period was $503 \text{ J} \cdot \text{cm}^{-2} \cdot \text{day}^{-1}$ and the rockwool slab was used for sweet pepper cultivation (Cultilene, Netherlands). The temperature in the glasshouse remained between 31°C and 12°C . The irrigation water was set to be 250 mL per plant per cumulative light intensity of $100 \text{ J} \cdot \text{cm}^{-2}$ between 6:00 am and 6:00 pm by the solar radiation integral control method. The composition of the nutrient solution initially supplied was K^+ 291, Ca^{2+} 246, Mg^{2+} 52, SO_4^{2-} 196, NO_3^- 1,147 and PO_4^{3-} 113 $\text{mg} \cdot \text{L}^{-1}$.

Soilless culture system and irrigation control

The closed-loop soilless culture system consisted of a drain collection tank, a nutrient solution tank, and an irrigation system (Fig. 1.2.1). The volume of the drainage collection tank was 4 L and the volume of water between the highest and lowest levels in the mixing tank was maintained at 3.6 L using the L1 level sensor and the L2 level sensor. The nutrient solution was supplied to the pressure-compensating button and the dripper by pump. As

the amount of nutrient solution accumulation increased by the irrigation program, the drainage generated was stored in the drainage collection tank connected to the bottom of the bed. When the irrigation program is completed at the end of the day, the solenoid valve at the bottom of the tank opens and the collected drainage is transferred to the mixing tank. The EC sensor (SCF-01A, DIK, Korea) installed in the mixing tank measures the EC in the tank and when it is $2.69 \text{ dS} \cdot \text{m}^{-1}$ or more, it opens the solenoid valve (V1) for 2 seconds and mixes it for 150 seconds to $2.69 \text{ dS} \cdot \text{m}^{-1}$ or less. At the end of the dilution process, EC $2.6 \text{ dS} \cdot \text{m}^{-1}$ was added and the supply was stopped when the highest level was detected by the L2 sensor. Mixed nutrient solution was used for next day irrigation. Data logger (CR1000, Campbell Scientific, USA) was used for the measurement and control in the drainage and nutrient mixing process.

Nutrient replacement cycle treatment and ion analysis

Replacement cycles of the recycled nutrient solution in the mixing tank were set to 1 week, 2 weeks, 3 weeks, and 4 weeks, respectively. Two closed-loop soilless culture system were used for each treatment. Three sweet pepper were grown on each system. The concentration of K^+ , Ca^{2+} , Mg^{2+} , Na^+ , SO_4^{2-} , NO_3^- , and PO_4^{3-} , Cl^- was analyzed weekly by measuring the pH of the nutrient solution in each mixing tank. The ICP-730 ES (Varian, USA) was used as the cation-inductively coupled plasma emission spectrophotometer. The concentration of anions was analyzed using an ion chromatograph (ICS-3000, Dionex, USA).

Ion activity and EC contribution ratio estimation

The ratio at which each ion contributes to the nutrient solution EC is determined by the equivalence and mobility of the ions. It was assumed that at the low nutrient concentration condition such as soilless culture system, the ratio of the equivalent ionic conductivity is the same as the ratio of the infinite ionic equivalent conductivity Eq. 1-2.

$$EC_i = \frac{|z_i|T_i\lambda_i}{\sum|z|T\lambda} \cong \frac{|z_i|T_i\lambda_{\infty}}{\sum|z|T\lambda_{\infty}} \quad Eq. 1-2$$

where EC_i is EC contribution ratio of an ion, T_i is ion activity, λ_i is ionic equivalent conductivity λ_{∞} is infinite ionic equivalent conductivity, i is ion. For more detailed analysis, the ionic activity except for the ion pair was used as the concentration contributing to the EC, and the ionic activity was calculated using the numerical analysis in the chemical equilibrium state in the solution (Adams, 1977). The radius and the infinite ionic equivalent conductance of the ions used are shown in Table 1.2.1. The chemical equilibrium state was obtained by substituting C_i for the initial value of A_i to obtain the ionic strength (I) of the nutrient solution Eq. 1-3, and then calculating the activity coefficient r_i from the equation of Debye-Hückel (Eq. 1-4). The activity (T_i) of each ion is the product of the molarity of the ion and the activity coefficient Eq. 1-5. The ion pairs were calculated using the activity and the equilibrium constant (K_{ij}) of each ion, and then A_i was obtained Eq. 1-6. This process was repeated until equilibrium was reached,

assuming that Cl^- and NO_3^- did not form cations and ion pairs, and that PO_4^{3-} existed in the form of H_2PO_4^- .

$$I = \frac{1}{2} \sum (A_i \cdot z_i^2) \quad \text{Eq. 1-3}$$

$$-\log r_i = \frac{Az_i^2\sqrt{I}}{1 + B\bar{a}_i\sqrt{I}\sqrt{I}} \quad \text{Eq. 1-4}$$

$$T_i = r_i A_i \quad \text{Eq. 1-5}$$

$$A_i = C_i - \sum \frac{T_i T_j}{K_{ij}} \quad \text{Eq. 1-6}$$

where z_i is ion charge, I is ionic strength, A and B are 0.509 and 0.329 (1atm, 25°C), respectively, \bar{a}_i is effective ion radius (\AA), A_i is molarity of the ion in the chemical equilibrium state, r is activity coefficient, T_i is ion activity (mole/L), C_i is estimated molarity, K_{ij} is equilibrium constant, i is ion, j means each ion having an opposite polarity to i .

Factors influencing the ionic concentration of reusable nutrients are primary nutrient absorption by plants. In this experiment, there was no difference in ion concentration, balance, and EC contribution during the 4 - week circulation hydroponic cultivation treatment. This suggests that the nutrient uptake of crop nutrients for four weeks in circulating sweet pepper has a relatively small effect on the nutrient content of reusable juice and may be influenced by drainage rate. Therefore, when the EC-based closed-loop soilless culture is performed within 4 weeks, it is considered that the distortion of the measured value due to the change of the ion concentration and the EC contribution is small.

Table 1.2.1. Effective ion radius and limit equivalent ionic conductivity.

	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	NO_3^-	SO_4^{2-}	$H_2PO_4^-$	Na^+	Cl^-
Ion radius (\AA)	3.0	6.0	8.0	2.5	3.0	4.0	4.5	4.5	3.0
Limit equivalent ionic conductivity ($\Omega \cdot \text{cm}^2 \cdot \text{mol}^{-1}$)	73.5	59.5	53.5	73.6	71.5	80.0	36.0	50.1	76.4

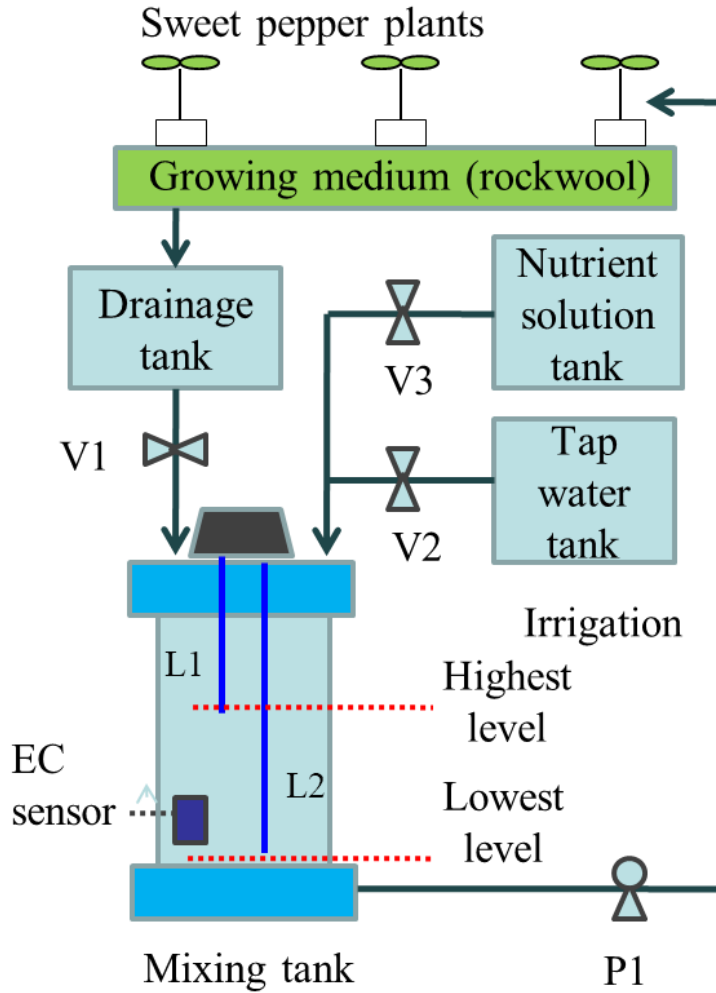


Fig. 1.2.1. A diagram of EC-based closed-loop soilless culture module used in the experiment (L1 and L2 = water level sensors, P1 = pump, V1, V2, and V3 = solenoid valves).

RESULTS AND DISCUSSION

Nutrient concentration changes according to nutrient solution replacement period

Equivalent concentrations of individual ions in the mixing tanks of each treatment varied with time (Fig. 1.2.2). In this experiment, the change of the concentration of individual ions according to the replacement cycle did not show any difference in the treatments within 4 weeks. In the case of K^+ , the initial nutrient concentration was $7 \text{ meq} \cdot L^{-1}$, and the concentration of total treatment was maintained at about $5 - 8 \text{ meq} \cdot L^{-1}$. The initial concentration of Ca^{2+} was $12 \text{ meq} \cdot L^{-1}$, and the concentration varied between about $11 - 14 \text{ meq} \cdot L^{-1}$. Mg^{2+} and Na^+ concentrations were relatively narrow and the initial concentrations were maintained between $2.0 - 2.7$ and $0.5 - 0.6 \text{ meq} \cdot L^{-1}$ at $2, 0.5 \text{ meq} \cdot L^{-1}$, respectively. In the anions, NO_3^- and SO_4^{2-} , PO_4^{3-} , and Cl^- started at the initial concentrations of 19, 4, 4 and 0.3, respectively, and were 14-19, 4-5, 1-4, and 0.3-0.5 $\text{meq} \cdot L^{-1}$, respectively. Ion concentration in the mixing tank was not clearly changed according to the replacement cycle. This could mean that other factors besides the replacement cycle may have an effect on the concentration change. The drainage ratio affects the EC of the drain (Corwin et al., 2007; Ahn et al., 2010), and reuse of this drain will affect the concentration of the nutrient solution in the mixed tank and the concentration of each ion. In particular, the volume and concentration of drainage in EC-based closed-loop soilless culture system is an important factor in determining the rate of dilution of raw water and the rate of input of new nutrients (Savvas and Mannos, 1999). The fact that there is no significant difference in growth or yield in closed-loop soilless culture system for a certain

period of time indicates that a period of time is required until the imbalance of nutrients is observed as significant effect (Raviv et al., 1998; Ehret et al., 2005). In this experiment, no significant difference was observed between the 1-week replacement treatment and the 4-week-interval replacement treatment because the sensitivity of ion concentration changes in the nutrient solution were relatively small for drainage reuse in the 4-week cycle.

Nutrient ratio changes according to nutrient solution replacement period

In order to understand the unbalance of ions due to the reuse of the nutrient solution, the ratio changes between ions were examined (Fig. 1.2.3). The ratio of $K^+ : Ca^{2+} : Mg^{2+}$ in the cation and $NO_3^- : PO_4^{3-} : SO_4^{2-}$ in the anion was determined. The ratio of cations in the initial nutrient solution was 0.31 : 0.51 : 0.18 in $K^+ : Ca^{2+} : Mg^{2+}$, and the ratio changed with the lapse of the treatment period. The ratio of each treatment did not show any significant difference, but the ratio of total treatments was distributed around Ca^{2+} and K^+ . The ratio of anions in the initial nutrient solution was 0.70 : 0.14 : 0.16 for $NO_3^- : PO_4^{3-} : SO_4^{2-}$. The ratio of anions and SO_4^{2-} and PO_4^{3-} were distributed in all treatments. Marti and Mills (1991) found that the absorption of K^+ was relatively high in the experiment of sweet pepper growth, while the absorption of Mg^{2+} was relatively low in Ca^{2+} . In this experiment, changes in the ratio of cations to that of K^+ and Ca^{2+} are mainly due to the nutrient absorption characteristics of sweet pepper.

Change in activity coefficient of each ion and estimation of EC contribution

The activity coefficients of monovalent ions such as K^+ , NO_3^- and $H_2PO_4^-$ and divalent ions such as Ca^{2+} , Mg^{2+} and SO_4^{2-} in the nutrient solution were maintained between 0.8 and 0.9 and 0.5 to 0.6, respectively, while maintaining EC 2.6 dS m^{-1} . And the activity coefficients of each ion remained constant with time (Fig. 1.2.4). The difference in the activity coefficients of valency of 1 and 2 is due to the ionic strength of each ion and the effective ionic radius of each ion. The divalent ion has a large effective ionic radius (Table 1.2.1) (Adams, 1977). Therefore, when the divalent ions of Ca^{2+} , Mg^{2+} , and SO_4^{2-} remain in the nutrient solution, the ionic strength is increased and the activity is decreased. In addition to these methods, models for estimating the ion activity coefficient by various methods have been proposed (Pazuki and Rohhani, 2006).

The ratio of each ion contribute to EC in the nutrient solution was highest in K^+ and NO_3^- , followed by Ca^{2+} , SO_4^{2-} , Mg^{2+} (Fig. 1.2.5). This is because the ratio of K^+ , NO_3^- , and Ca^{2+} in the initial nutrient solution was high. In the case of Ca^{2+} , however, because the bivalent ion has extremely low infinite equivalent ionic conductivity and the activity coefficient, the EC contribution rate was low. In fact, when the EC control by the nutrient supply of certain components is carried out, if the absorption of the monovalent ions and the persistence of the divalent ions continue, even the low monovalent ions have a high EC contribution, and even the high divalent ions have the underestimated EC contribution (Son, 1998). Therefore, irregularity of the ion component accompanied by reduction of monovalent ions and increase of divalent ions can be accelerated by the nutrient solution supplied irrespective of the change of the ion component in the nutrient solution.

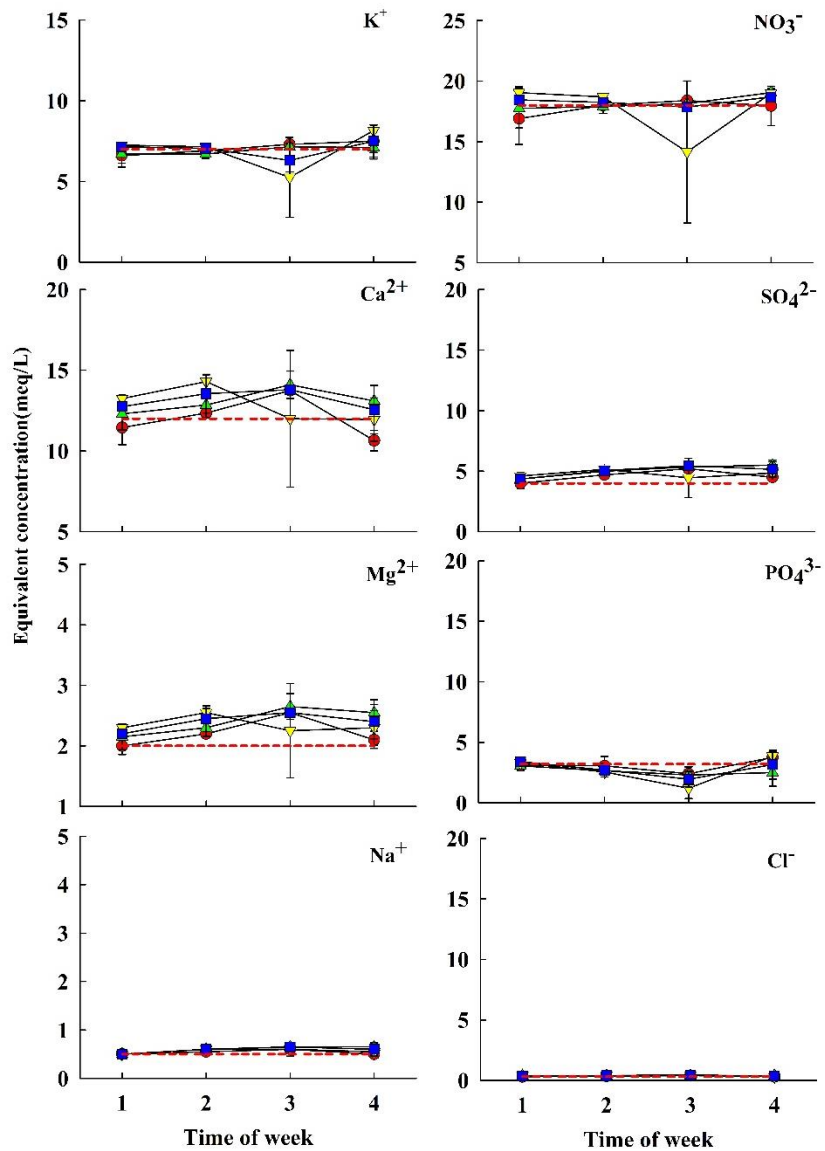


Fig. 1.2.2. Change in individual ion equivalent concentrations of nutrient solutions in EC-based closed-loop soilless culture of sweet pepper (● 1- week, ▲ 2- week, ▼ 3- week, ■ 4- week renewal intervals, and --- $n=2$ mean initial ion equivalent concentration before the circulation started).

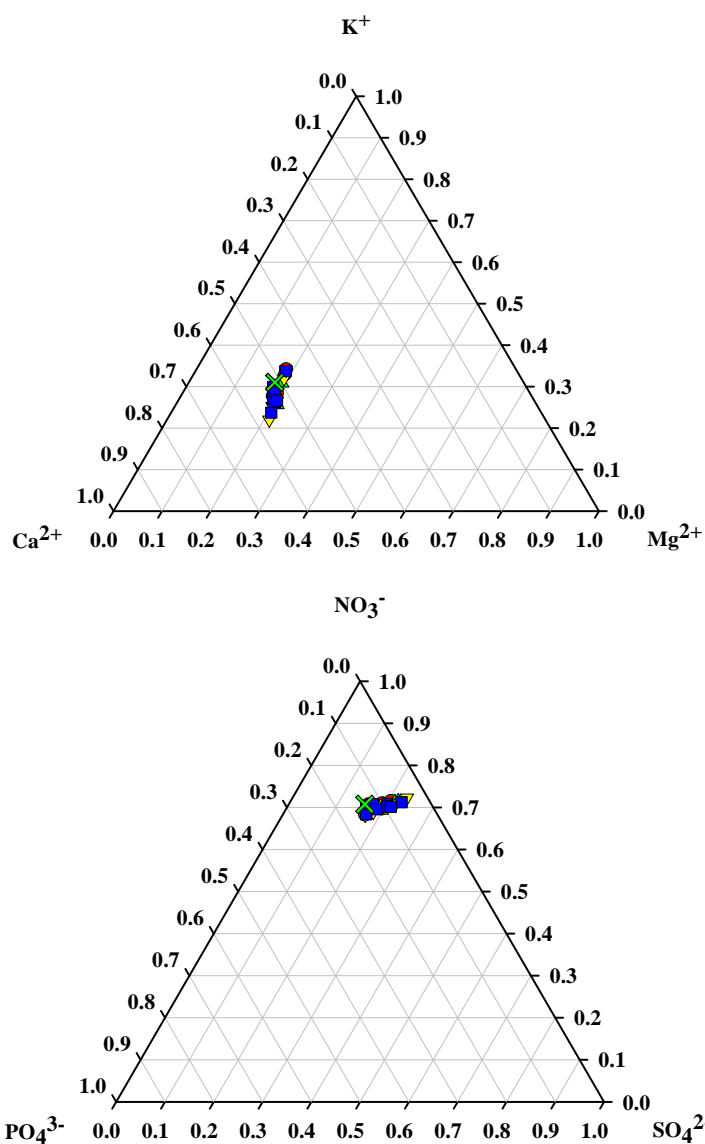


Fig. 1.2.3. Change in cation (top) and anion (bottom) balances of the nutrient solution in EC-based closed-loop soilless culture of sweet pepper at different renewal intervals (\times initial ratio, ● 1- week, ▲ 2- week, ▼ 3- week, and ■ 4- week renewal intervals).

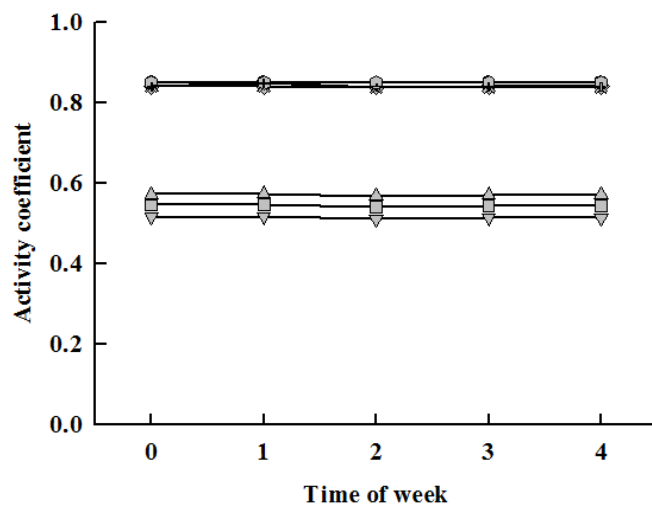


Fig. 1.2.4. Change in individual activity coefficients of ions in the nutrient solution (◇

K^+ , ■ Ca^{2+} , △ Mg^{2+} , × NO_3^- , ▽ SO_4^{2-} , ● $H_2PO_4^-$, and + Cl^-).

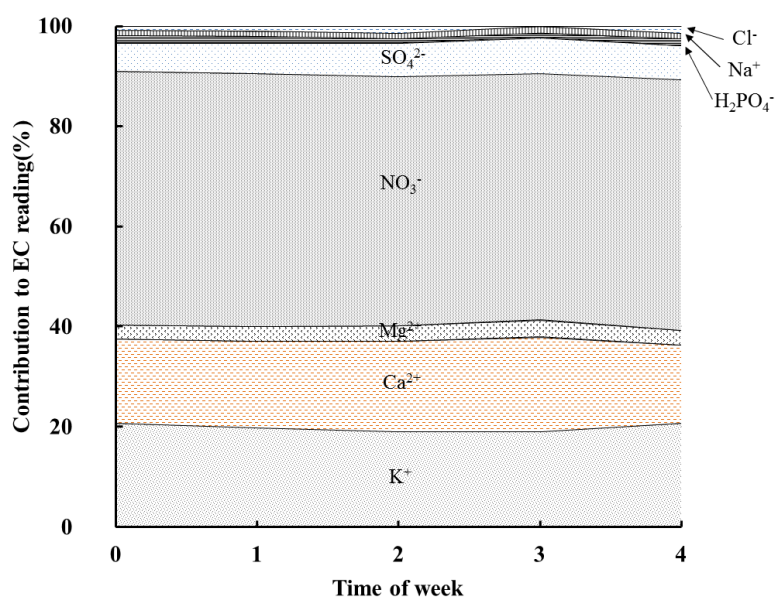


Fig. 1.2.5. Change in individual ionic contributions to EC reading in the nutrient solution.

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CHAPTER 2

Theoretical and Experimental Analyses for Steady-state Nutrient Control in Soilless Cultures

ABSTRACT

Soilless culture system is advantageous for variable control. However significant nutrient fluctuations may occur in soilless culture system. This is a very unfavorable condition in terms of consistency of treatments or experimental conditions in plant studies and plant production performance in agricultural system. The objectives of this study were to establish theoretical models to analyze nutrient variations in closed soilless cultures and to theoretically derive technique for the steady-state control of nutrients. Mathematical models presenting soilless culture systems were constructed and greenhouse experiments for investigation on behavior of nutrient fluctuations and parameter estimation were carried out using four soilless culture modules. Sweet peppers were grown for 12 weeks under electrical conductivity (EC) based automated soilless culture system. Unlike the dynamic change in nutrient concentration, the ratio between nutrients showed a certain deterministic tendency in the experimental analysis. Scenario for control was applied to the theoretical analysis: long-term feedback of nutrient ratio changes was conducted. Through the theoretical analyses, the nutrient balance convergence on the target value by

long-term feedback was confirmed and quick transition of individual nutrient concentration to steady-state under steady-state control of EC was theoretically predicted.

Additional Keywords: Michaelis-Menten equation; nutrient uptake model; nutrient use efficiency; plant mineral nutrition; soilless culture; closed-loop; steady state control

INTRODUCTION

Soilless culture began with the scientific link between plants and mineral nutrients established by Liebig's law of the minimum (Hoagland and Arnon, 1950). This link also provided a reductive perspective for essential factors of plant nutrition in the root zone (Marschner and Marschner, 2012). With these theoretical and technological transitions, a more controllable cultivation platform for plant studies and agricultural production was established (Epstein and Bloom, 2005; Jones, 1982; Taiz and Zeiger, 2006).

Although soilless culture is advantageous for variable control, root zone nutrients may change more sensitively than in soil-based system. Therefore, significant nutrient fluctuations may occur in soilless culture system when appropriate management technique for nutrients is not applied (Noordwijk, 1990; Zekki et al., 1996). This is a very unfavorable condition in terms of consistency of treatments or experimental conditions in plant studies and plant production performance in agricultural system. Soilless culture is often used in plant studies where active management of the control variables is required (Epstein and Bloom, 2005; Taiz and Zeiger, 2006). In plant studies, specifications for nutrient conditions or nutrient treatment are limited, such as the initial nutrient concentration, the concentration of nutrient feed, and the nutrient supply rate (McDonald et al., 1996). However, fluctuations in nutrient concentrations in the root zone are largely affected by uptake concentration of plants (Le Bot et al., 1998; Noordwijk, 1990). The uptake concentration also changes dynamically according to the environmental conditions (Noordwijk, 1990). Therefore, it is difficult to interpret these conditions in a way that is compatible with other literature data (Ingestad and McDonald, 1989).

Therefore, even in the identical initial condition of the root zone nutrients, a significant difference in plant growth response can be observed under the condition that the nutrient variation is allowed (McDonald et al., 1996). On the other hand, when the initial concentrations of each treatment were maintained for the duration of the experiment, uniform results in plant growth were observed in various concentration treatment ranges (McDonald et al., 1996). In other words, it is difficult to conclusively interpret the results of the experiments under the condition that the nutrient variation is permitted because it involves the influence of the plant itself on the system over time. On the other hand, in the condition of steady state control of nutrients, the effect of nutrient is converted to the matter of nutrient supply rate and if the nutrient feed rate is maintained, the symptom of deficiency is not observed even at very low concentrations (Ingestad and McDonald, 1989; McDonald et al., 1996). In this case, the nutritional significance can be observed in perspective of a balance between nutrients rather than the concentration of nutrients (Steiner, 1980).

In the case of agricultural production, a closed-loop or semi-closed-loop soilless culture system that reuses discharged nutrients and water can minimize resource losses but is accompanied by nutrient variations resulting in nutrient imbalance and instability in crop production (Hao and Papadopoulos, 2002; Kläring, 2001; Raviv and Lieth, 2008; Zekki et al., 1996). Because of these constraints, the way of managing the nutrient fluctuations in soilless culture system thus far has been a form of replacement rather than a control technique, that is, an open-loop structure of nutrient supply that repeatedly discharges drainage at a certain proportion by excessively supplying fresh nutrient solution to root zone (Beerling et al., 2014; Noordwijk, 1990; Olympios, 1999; Savvas and Manos, 1999).

However, due to the intensive use of fertilizers and the repeated discharge of a certain percentage of drainage, the open-loop supply practices accompanies problems in agricultural sustainability and poses a serious threat to ecosystem enough to be included in regulations of national government (Beerling et al., 2014; Olympios, 1999).

Steady state nutrient control has already been implemented as an experimental level device (Ingestad and Lund, 1986). However, in most plant studies or agricultural systems applying soilless culture systems as a basic cultivation platform, nutrient management takes place at the general technical level of soilless culture. Therefore, the technique for nutrient control in general soilless culture system is expected to significantly contribute to the sustainability of the agricultural production system and the reproducibility of the results of the plant study.

However, soilless culture systems have been technical constraints in control of nutrients for specific nutrient conditions in root zone (Beerling et al., 2014). Due to these seemingly complicated aspects and the instabilities in plant production of the closed-loop soilless culture system, the approaches to nutrient management technique have been mainly focused on controlled experiments. Most of the experimental treatments have been applied by the manipulation of the terminal variables and the parameters such as the nutrient-reuse period (Ehret et al., 2005; Ko et al., 2013), EC control (Rouphael et al., 2016; Signore et al., 2016), semi-closed-loop systems (level of drainage discharge) (Massa et al., 2011; Rouphael et al., 2016), fertilizer compositions (Gent and Short, 2012; Hao and Papadopoulos, 2002; Neocleous and Savvas, 2015, 2018), and differences in irrigation systems (Bouchaaba et al., 2015; Incrocci et al., 2006; Zekki et al., 1996).

Basically, the nutrient uptake phenomenon of a plant follows the Michaelis-Menten equation (Clarkson, 1985; Cott et al., 2018; Le Bot et al., 1998). In the case of a system that follows this mechanism, it can have a steady-state solution according to the conditions of input variables and parameters (Golicnik, 2011), which means that the change in nutrient concentration in the plant root zone can converge to a specific value after a certain period of time. Furthermore, from the plant stoichiometric point of view, the accumulation of nutrients is explained as a chain of reactions depending on the biochemical composition of plants, so that in the ratio between nutrients, convergent changes are observed compared to the variation of individual nutrients (Ågren, 2008, 2004; Ågren et al., 2012; Kerkhoff and Enquist, 2006; Knecht and Göransson, 2004). These suggest that the plant has its own nutrient balance control ability and stability, and it means that there is a field that is confronted with the basic premise of studies on nutrient management thus far. These confronted perspectives might suggest that behind the complicated nutrient fluctuation, there could be the behaviors of the variables that follow a specific mechanism. However, there have been no attempts to analyze the nutrient management and control techniques in the soilless culture system in consideration of the abovementioned plant nutritional aspects. The objective of this study was to establish theoretical models to analyze nutrient variations in closed soilless cultures and to theoretically derive technique for the steady state control of nutrients.

MATERIALS AND METHODS

Description of soilless culture models

The models of the soilless culture system used in the simulation consist of water and nutrient transport, nutrient uptake, and nutrient solution mixing (Fig. 2.1). The basic structure of our model builds on previous models of soilless culture system (Raviv and Lieth, 2008; Silberbush et al., 2005; Silberbush and Ben-Asher, 2001).

The simulation model reflected the same structure and the nutrient solution mixing process of the electrical conductivity (EC)-based soilless culture system as that described in the soilless culture system section. The EC measurement in the real system is conducted for control of the total equivalent concentration in the nutrient solution based on the linear relationship between those two factors (Savvas and Adamidis, 1999). Based on this assumption, the EC measurement was replaced by the sum of the total equivalent concentrations in the simulation of nutrient solution mixing. For nutrients and water transport in the substrate, mass flow and diffusion models in porous media were applied (Corwin et al., 1993; Shackelford and Daniel, 1991; Snape et al., 2008). The nutrient uptake by plants is generally expressed by the Michaelis-Menten equation, by which the rate of nutrient uptake is mainly driven by concentration, but in this study, a modified model that has an additional variable for the transpiration rate was used. This model takes into account the reduction of the nutrient depletion zone around the roots due to the accelerated mass flow generated by an increase in the rate of transpiration with plant growth (Nomiya et al., 2013; Sago et al., 2011). Since transpiration is a variable that

includes parameters for plant growth changes (Baille et al., 1994; Ta et al., 2012), the model was used to express the change in the nutrients absorption rate with the growth of plants. The target nutrients for the simulation were selected as macronutrients in cations (K^+ , Ca^{2+} , Mg^{2+}), which are the major targets of nutrient balance control in soilless cultures (Bautista et al., 2009; Garcia et al., 1999; Neocleous and Savvas, 2013; Savvas and Gizas, 2002). The Michaelis-Menten constant used in the nutrient uptake model was estimated by performing a numerical integration-based progress curve analysis using the data of resource inputs and nutrient concentration measured in the experiment of a closed-loop soilless culture system, described in the Soilless culture system section. The mixing process of the nutrient solution described the Soilless culture system section was prepared by referring to the automated system for injection and mixing of the raw water, drainage, and stock solution of standard composition (Savvas, 2002; Savvas and Manos, 1999). Simulation and progress curve analysis of these models were performed using Berkeley Madonna 8.3.23 (Berkeley Madonna, Inc., University of California, Berkeley). The definitions, values, units, and sources of the parameters used in the models are summarized in Table 2.1.

Models for the soilless culture system

Water transport

Water transport in a closed-loop soilless culture system occurs between the substrate, the drainage tank, and the mixing tank, and these correspond to the Eqs. of 2-1, 2-2 and 2-3, respectively.

$$\frac{dV_{sub,n}}{dt} = \begin{cases} Q_{ir} - Q_{dr,1} - Q_{tr,1}, & n = 1 \\ Q_{dr,n-1} - Q_{dr,n} - Q_{tr,n}, & \text{and } n > 1 \end{cases} \quad Eq. 2-1$$

$$\frac{dV_{drg}}{dt} = Q_{dr,n} - Q_{drg} - Q_{disch} \quad Eq. 2-2$$

$$\frac{dV_{mix}}{dt} = Q_{drg} + Q_{stk} + Q_{wtr} - Q_{ir} \quad Eq. 2-3$$

where V is the volume of water, subscript sub , n is the water content of each layer when the substrate is divided into n layers, drg is the drainage tank, and mix is the mixing tank. Q is the flow rate of the water, subscript ir is the irrigation flow rate to the top of the substrate ($n = 1$), $dr, n - 1$ and dr, n are the flow rates of the drain from the $n - 1$ layer and to the $n + 1$ layer, respectively, and tr, n represents the transpiration rate of the substrate layer n . Here, $Q_{dr,n}$ is basically the difference between the irrigation rate to the substrate and the transpiration rate ($Q_{ir} - Q_{tr,1}$ or $Q_{dr,n-1} - Q_{tr,n}$) (Shin et al., 2014). However, $Q_{dr,n}$ and $Q_{tr,n}$ are restricted by the field capacity (F) and difficult available water (W_{DAW}), respectively, for the volume of the layer of the substrate (S_{sub}), and the flow for $Q_{dr,n}$ occurs only when $V_{sub,n} > S_{sub}F$, and $Q_{tr,n}$ flows only when $V_{sub,n} > S_{sub}W_{DAW}$. Q_{drg} , Q_{stk} , and Q_{wtr} , correspond to the flow rates of drain, stock solution, and raw water, respectively, introduced into the mixing tank. Q_{disch} is a variable that is applied to the semi-closed-loop system, meaning the flow rate of the nutrient

solution discharged to the outside when the volume of stored drainage (V_{drg}) exceeds the capacity of the drainage tank (S_{drg}).

Nutrient transport

In a soilless culture system, the movement of nutrients follows the same path as the movement of water, and the movement of nutrients between substrate, drainage tank, and mixing tank can be expressed as Eqs. 2-4, 2-5, and 2-6, respectively.

$$V_{sub,n} \frac{dC_{sub,n}^I}{dt} = \begin{cases} Q_{ir}C_{mix}^I - Q_{dr,1}C_{sub,1}^I - J_1^I - \frac{D^I A(\theta_1 C_{sub,1}^I - \theta_{n+1} C_{sub,n+1}^I)}{Z}, & n = 1 \\ Q_{dr,n-1}C_{sub,n-1}^I - Q_{dr,n}C_{sub,n}^I - J_n^I - \frac{D^I A(\theta_n C_{sub,n}^I - \theta_{n+1} C_{sub,n+1}^I)}{Z} - \frac{D^I A(\theta_n C_{sub,n}^I - \theta_{n-1} C_{sub,n-1}^I)}{Z}, & n > 1 \end{cases} \quad Eq. 2-4$$

$$V_{drg} \frac{dC_{drg}^I}{dt} = Q_{dr,n}C_{sub,n}^I - Q_{drg}C_{drg}^I - Q_{disch}C_{drg}^I \quad Eq. 2-5$$

$$V_{mix} \frac{dC_{mix}^I}{dt} = Q_{drg}C_{drg}^I + Q_{stk}C_{stk}^I - Q_{ir}C_{mix}^I + Q_{wtr}C_{wtr}^I \quad Eq. 2-6$$

where C represents the equivalent concentration of nutrients, and superscript I is the type of ions (K^+ , Ca^{2+} , Mg^{2+}). Subscripts sub, drg, mix, stk , and wtr represent the source of the nutrients and mean substrate, drainage tank, mixing tank, stock solution, and

raw water, respectively. J^I means uptake rate of nutrients (see Nutrient uptake section). The final term of Eq. 2-4 is applied, referring to the diffusion of solutes in soil, where D is the diffusion coefficient of an ion of I , A is the cross-sectional area of the substrate; θ_n is the water content of the n -layer ($V_{sub,n}/S_{sub}$); and Z is the height of the n -layer. In the case of the last layer, the diffusion term from $n + 1$ is excluded, since only diffusion from the previous layer occurs.

Nutrient uptake

The nutrient uptake rate of the plant in the substrate is a function of Michaelis-Menten, which can be expressed as Eq. 2-7.

$$J_n^I = \frac{J_{max}^I C_{sub,n}^I}{K_m^I + C_{sub,n}^I} \quad \text{Eq. 2-7}$$

where J_{max}^I means the maximum absorption rate of the ion, and K_m^I is the Michaelis-Menten constant. J_n^I is the absorption rate of individual nutrient I in layer n , and the equation applying the change in the rate of absorption of nutrients to the increase of transpiration rate ($Q_{tr,n}$) is shown in Eq. 2-8.

$$J_n^I = \frac{J_{max}^I C_{sub,n}^I Q_{tr,n}}{K_m^I + C_{sub,n}^I Q_{tr,n}} \quad \text{Eq. 2-8}$$

Determination of the mixing ratio

Q_{stk} , Q_{drg} , and Q_{wtr} of the semi-closed-loop soilless culture system are determined by Eqs. 2-9, 2-10, and 2-11, respectively.

$$Q_{stk} = \begin{cases} Q_{ir} - Q_{wtr} - Q_{drg}, & \text{and } V_{drg} > 0 \\ \frac{Q_{ir}(C_{tar} - \sum C_{wtr}^I)}{\sum C_{stk}^I - \sum C_{wtr}^I}, & \text{and } V_{drg} \leq 0 \end{cases} \quad Eq. 2-9$$

$$Q_{drg} = \begin{cases} \frac{Q_{ir}(C_{dil} \sum C_{stk}^I - \sum C_{stk}^I \sum C_{wtr}^I - C_{dil}C_{tar} + C_{tar} \sum C_{wtr}^I)}{\sum C_{stk}^I \sum C_{drg}^I - \sum C_{stk}^I \sum C_{wtr}^I - C_{dil} \sum C_{drg}^I + C_{dil} \sum C_{wtr}^I}, & \text{and } V_{drg} > 0 \\ 0, & \text{and } V_{drg} \leq 0 \end{cases} \quad Eq. 2-10$$

$$Q_{wtr} = \begin{cases} \frac{Q_{drg} \sum C_{drg}^I - Q_{drg} C_{dil}}{C_{dil} - \sum C_{wtr}^I}, & \text{and } V_{drg} > 0 \\ Q_{ir} - Q_{stk}, & \text{and } V_{drg} \leq 0 \end{cases} \quad Eq. 2-11$$

where C_{dil} is the target equivalent concentration for the dilution of the total nutrients in the drainage, and C_{tar} is the target equivalent concentration for the total nutrients in the nutrient solution to be supplied to the plant. Q_{stk} , Q_{wtr} , and Q_{drg} for the closed-loop system are determined by the Eqs. 2-12, 2-13 and 2-14, respectively, and the calculation of the case where there is no drainage to be used ($V_{drg} \leq 0$) is the same as in the Eqs. 2-9, 2-10, and 2-11. The open-loop system is always applied in the case of $V_{drg} \leq 0$ in Eqs. 2-9, 2-10, and 2-11.

$$Q_{stk} = \frac{Q_{ir}(C_{tar} - \sum C_{wtr}^I) - Q_{drg}(\sum C_{drg}^I - \sum C_{wtr}^I)}{\sum C_{stk}^I - \sum C_{wtr}^I} \quad Eq. 2-12$$

$$Q_{wtr} = Q_{ir} - Q_{drg} - Q_{stk} \quad Eq. 2-13$$

$$Q_{drg} = Q_{dr,n} \quad Eq. 2-14$$

Theoretical analysis and simulation conditions

For theoretical analysis, the nutrient balance changes were investigated in relation to the degree of nutrient injection by the open-, semi-closed-, and closed-loop systems. The variation in the rate of transpiration in each system was estimated by measured water amounts injected in the closed-loop system during the experimental period. For derivation of nutrient control technique, simulation scenario was applied: the ratio of nutrients was controlled by long-term feedback, assuming that the ratio changes relative to the nutrient concentrations are relatively stable and convergent.

The transpiration rate, one of main disturbance factors of root-zone nutrient change, was changed through a random walk, and the nutrient control was performed by changing the ratio of nutrients in the stock solution for nutrient replenishment under these conditions. The maximum fluctuation range during the cultivation period in this study was applied for the range of the random walk changes. In the mixing process of the nutrient solution, the inflow rate of the stock solution was set to be constant, and the inflow rate of raw water was controlled for constant volume of water storage. The feedback period was set to 12

weeks, which is the total period of the simulation. After the end of each simulation, the difference between the average value of the simulated nutrient ratio and the target value of each nutrient ratio in the substrate was fed back to adjust the ratio of each nutrient in the stock solution for integral feedback control.

Experimental analysis

Soilless culture systems

The structure of a general soilless culture system is composed of a mixing tank, substrates for plant cultivation and a drainage tank, and nutrients and water are circulated for reuse or passed through the system and discharged to the outside according to a command from the controller unit (Raviv and Lieth, 2008). Nutrient control is based on the EC measurements of nutrient solutions for irrigation in mixed tanks. In the case of open-loop soilless culture, the nutrient solution in the mixing tank is supplied to the plant according to the irrigation order of the controller unit, and the stock solution of standard composition is injected into the mixing tank to match the EC of water in the tank to the target value, and drains the nutrient solution from the substrates discharged outside the system. Basically, the main aim of water and stock solution supply into closed-loop or semi-closed soilless culture system is the replenishment of the water removed by transpiration and the nutrients absorbed by plant. For this purpose, total collected drainage is transferred to the mixing tank, and the input amount of water and stock solution is adjusted to control the EC variation in the mixing tank to the target value (Savvas and Manos, 1999). In semi-closed-loop soilless culture system, the drainage is diluted to a

value lower than the target EC for irrigation supply by mixing the drainage and raw water according to the user's setting, which is the most popular method among nutrient solution reuse systems, and a stock solution is injected to control EC in the mixing tank to the target value(Savvas, 2002). However, the drainage reuse ratio is lower than that of the closed-loop system; thus, unused drainage is accumulated and discharged outside the system when the storage capacity has been exceeded.

Description of the mixing module

The experimental nutrient-mixing module consists of a drainage tank, a nutrient solution tank, and a standard nutrient solution tank (Fig. 2.2). In the drainage tank, the weight of the collected drainage and EC are measured. In the mixing tank, nutrient solution mixing is carried out according to the mixing ratio, which is determined according to the treatment of the experiment. The storage capacity of the drainage tank was 11.7 L, and the capacity of the mixing tank was 19.4 L. The feed amount of drainage, raw water, and standard nutrient solution into the mixing tank were adjusted to the measured value of the ultrasonic level sensor (UHA-300, Unics, Korea) installed at the top of the mixing tank. A pyranometer (SP-110, Apogee, USA) was used for the solar radiation-based irrigation control. A data logger (CR1000, Campbell Scientific, USA) and controller (SDM-CD16AC, Campbell Scientific, USA) were used for the measurement and control in the overall mixing process.

Cultivation experiment

The cultivation experiment using the nutrient solution mixing module was carried out in a Venlo-type greenhouse located at the experimental farm of Seoul National University (Suwon, Korea, Lat. 37.3° N, long. 127.0° E). Daytime temperature, nighttime temperature, and relative humidity of the greenhouse remained between 25-30 °C, 15-22 °C, and 50-80%, respectively, by an environmental control system of the greenhouse. Sweet pepper (*Capsicum annuum* L. 'Fiesta') plants were used in the experiment and seeded on July 15, 2011. The sweet pepper plants were transplanted into 90 cm (L) × 15 cm (W) × 7 cm (H) rockwool slabs (Cultilene, the Netherlands) on September 29, 2011. Three sweet pepper plants were planted per slab, and three slabs were used per experimental treatment. The planting density was 2.8 plants/m². The modules for the treatment of the mixing nutrient solution were composed of four modules: open-loop (OL), closed-loop (CL), and semi-closed-loop modules with two EC levels for a drainage dilution of 0.65 and 1.3 dS·m⁻¹, respectively (SCL 0.65 and SCL 1.3). The determination of the mixing ratio is based on the calculation procedure described in Determination of the mixing ratio section. A solar radiation-based irrigation control was applied, and basically, when the cumulative radiation amount reaches 100 J · cm⁻², 150 mL of the nutrient solution is supplied per plant. However, the irrigation amounts of nutrient solution per plant were adjusted by the meteorological condition so that a drainage ratio of approximately 30% is maintained. Compositions of the standard nutrient solution used in this experiment were 14.56 NO₃⁻, 1.18 H₂PO₄⁻, 5.96 K⁺, 9.56 Ca²⁺, and 3.38 Mg²⁺ (in meq·L⁻¹). The target EC of the nutrient solution mixing module was set to 2.6 dS · m⁻¹, and the EC of the raw water was set to 0.15 dS · m⁻¹.

The sweet pepper plants were cultivated by open-loop nutrient supply until December 15, 2011, and after that, each treatment was applied, and the experiment was finished on March 9, 2012. On the day before the initiation of the treatments, a large amount of a standard nutrient solution was irrigated in an open-loop nutrients supply in order to make the initial condition of the nutrients balanced in the rockwool slab of all treatments close to the standard composition. The total equivalent concentration required for the mixing ratio calculation of nutrient solution was estimated using Eq. 2-15, which is a function of the measured EC.

$$C_t = a \cdot EC - b \quad \text{Eq. 2-15}$$

Eq. 2-15 is an empirical formula estimated from various compositions and concentrations of nutrient solutions, and the EC of nutrient solutions for soilless culture and used for the automation of nutrient solution preparation in an EC-based closed- or semi-closed-loop system (Savvas, 2002; Savvas and Manos, 1999). C_t is the total equivalent concentration, and a and b are the conversion coefficients given by Savas and Adamidis (1999).

Nutrients analyses and statistics

To observe the changes in the ratio between nutrients in the root zone of the soilless culture system, samples of nutrient solution in the rockwool slabs were extracted using a syringe. The collection points of the nutrient solution in the rockwool slab were randomly

selected for the collection of representative samples of the overall concentration in the root zone. Then, 10 mL of a root zone nutrient solution was collected for each extraction, and this was performed 5 times to make a 50 mL sample. Six samples per treatment were collected each time, every two weeks. K^+ , Ca^{2+} , and Mg^{2+} were analyzed using an inductively coupled plasma optical emission spectrometer (ICP-730ES, Varian, Australia). The SAS system (version 9.2, SAS Institute, USA) was used for statistical analysis.

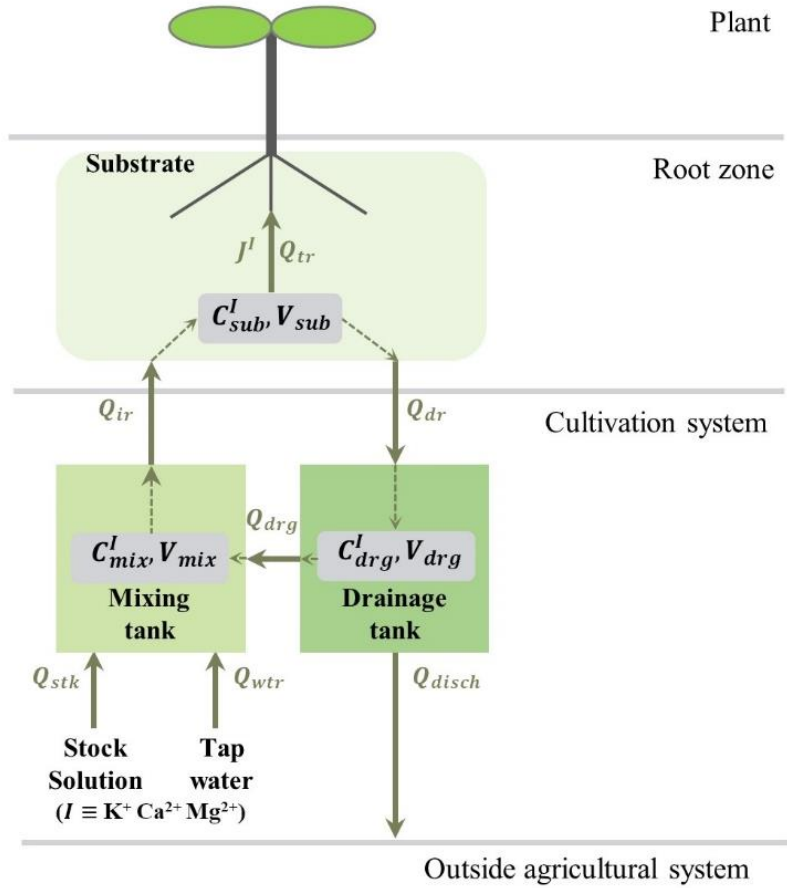


Fig. 2.1. Schematic description of open-, semi-closed-, and closed-loop soilless culture systems in the simulated condition. Nutrients (I) from the stock solution (Q_{stk}) and tap water (Q_{wtr}) are circulated through the mixing tank, substrate, and drainage tank by the flow rate of irrigation (Q_{ir}), drainage (Q_{dr}), drainage injection (Q_{drg}) or are discharged (Q_{disch}) outside agricultural system. The water and nutrients are consumed by transpiration rate (Q_{tr}) and nutrient uptake rate of plant (J^I), respectively. C and V are nutrient concentration of I and volume of water mixing tank (mix), substrate (sub), and drainage tank (drg), respectively.

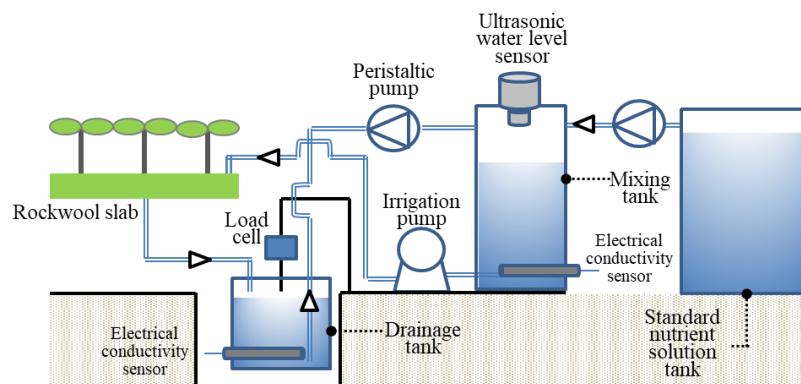


Fig. 2.2. Schematic description of a nutrient solution mixing module

RESULTS AND DISCUSSION

Experimental analysis

Model verification in the greenhouse experiment

In the simulation of a closed-loop soilless culture system (CL) for the verification of the nutrient uptake parameters estimated by the progress curve analysis, changes in the nutrient concentration in the substrate were expressed as RMSE 2.96-6.36, R^2 value at the level of 0.81-0.88, and overall, similar trends were observed (Fig. 2.3a). The nutrient ratios were simulated at RMSE of 0.0161-0.0188 and an R^2 value of 0.72-0.94. The changes in the nutrient ratio were clearly distinguished from that of the concentration; Ca^{2+} and Mg^{2+} were increased, and K^+ was decreased (Fig. 2.3b). The increase in divalent ions such as Ca^{2+} and Mg^{2+} in a closed-loop soilless culture is generally observed (Sonneveld and Voogt, 2009). This can be seen as the result of the selective uptake of nutrients by the plants (Steiner, 1980), and this can be considered the model of the soilless culture system constructed in this study and the nutrient solution mixing modules designed for the experiment to reflect these effects appropriately.

As explained in Materials and Methods, the amount of nutrients input in the open-loop, semi-closed-loop, and closed-loop soilless culture system is determined in different ways, and the amount of nutrients input into the system is a major factor in the change in nutrient concentration. Therefore, we compared the cumulative nutrients input in the greenhouse experiment with simulated results to verify the normal performance of nutrient input calculation in the soilless culture model (Fig. 2.4). In the greenhouse experiment, the

amounts of nutrients input were increased in the order of CL, SCL 1.3, SCL 0.65 and OL, according to the nutrient solution mixing treatment. The simulated results were in proportion to the cumulative nutrient inputs of the experiment with the overall R^2 value of 0.99. For the individual treatments of CL, SCL 1.3, SCL 0.65, and OL as 0.95, 0.99, 0.99 and 0.99, respectively. Therefore, it can be seen that the nutrient solution mixing process in the simulation is also performed normally.

Changes on nutrient concentration and ratio in the experiment

Changes in nutrient concentration in the substrate were similar throughout the experiment period, and significant differences between the treatments were not consistently observed (Fig. 2.5a). On the other hand, when the nutrients were converted to a ratio between the nutrients, the tendency of the change was clearly distinguished, and a relatively consistent difference was observed between the treatments (Fig. 2.5b).

The ratio of K^+ in CL, which has the least nutrient input, was significantly lower than other treatments and deviated most from the initial value, and OL, which has the highest nutrient input, was the closest to the initial value. In the CL treatment, Mg^{2+} and Ca^{2+} remained relatively higher than other treatments after the observation of significant differences in 4 and 8 weeks after treatment, respectively, and were significantly lower in OL and tended to be the closest to the initial value (Fig. 5b). Thus, unlike nutrient concentrations that exhibited similar overall trends, the tendency to decrease K^+ and to increase Ca^{2+} and Mg^{2+} were clearly distinguished. Furthermore, in the case of K^+ , the equilibrium state was observed after the 8th week of the experiment in CL and in Mg^{2+} and

after the 6th week of the experiment in all treatments. The ratio between the nutrients showed specific trends according to open-loop, two levels of the semi-closed-loop, and closed-loop soilless culture system, under the greenhouse experiment conditions exposed to dynamic environmental change. These results suggest that the changes in nutrient ratios are determined by the dominant function of a specific mechanism rather than being interpreted as the unstable characteristics of nutrient management due to nutrients recycling. On the other hand, the nutrient concentrations were relatively difficult to clearly distinguish between the treatments and the nutrients as described above, and an overall gradual increase was observed from the 8th week onwards (Fig. 5a). Changes in nutrient concentrations in the root zone in soilless culture systems vary depending on the difference between irrigated nutrient concentration and nutrient uptake concentration (Noordwijk, 1990). The change in the uptake concentration is mainly affected by variations in the transpiration rate (Le Bot et al., 1998), and in this experiment, the transpiration rate per plant was gradually increased, with fluctuation in the latter half of the experiment (Fig. 2.7a), and it is considered that the fluctuations affected the nutrient uptake concentration; additionally, corresponding changes of nutrient concentration in the substrate were observed in the experiment. The tendency for similar changes in the concentration of each nutrient and treatment can be seen as characteristic of an EC-based soilless culture system, which controls the total nutrient concentration. Regardless of the treatment, as described in Materials and Methods, the EC representing the total equivalent concentration of the irrigated nutrient solution was always controlled to the same target value and was supplied to the substrates, so that overall similar changes in each nutrient could be observed.

Although significant differences were observed according to treatments, there were no consistent differences between Ca^{2+} and Mg^{2+} , and K^+ repeatedly fluctuated until the 6th week; thereafter, the CL system, which has the highest drainage reuse ratio, showed a distinct change from the other treatments (Fig. 2.5a). The tendencies observed in the changes of the nutrient ratio in Figure 2.5b mean that the influence of the fluctuation in nutrient uptake concentration can be minimized when the nutrient concentration is converted into the relative ratio. The nutrient uptake concentration is the ratio of nutrient uptake to the water uptake by the plant, and the water uptake corresponds to the common denominator of each nutrient uptake concentration (Noordwijk, 1990). Therefore, it can be interpreted that when the fluctuations of nutrient concentration in the root zone are expressed as relative ratios, the effect of the variation in evapotranspiration is minimized, the tendency according to the uptake ratio difference of each nutrient is observed, and a simplified analysis platform can be provided.

Theoretical analysis

Nutrients balance in the closed-, semi-closed, and open-loop soilless culture systems

The deviation between the final value of the nutrient ratio in the substrate and the nutrient ratio in the standard nutrient solution and discharged nutrients to the outside the system is varied with the amount of nutrients input according to the application of open-loop, semi-closed-loop, and closed-loop systems (Fig. 2.6). However, when the deviations in the ratios were summarized for the amounts of nutrients inputs in the x-axis, certain trends in the deviation decreases were observed, and similar tendencies were confirmed by

the results from the greenhouse experiment. Unstable changes in nutrient balance with increasing levels of nutrient reuse or nutrient emission levels were not observed. Each symbol in the graph represents different nutrient management system that has been discretely separated from each other, but it can be seen that continuous changes are observed according to the nutrients inputs from the systems.

Thus, it can be interpreted that the closed (or semi-closed)-loop soilless culture system, which is distinguished from the open-loop system in terms of plant nutrition, has differences in the process of nutrients and water transport; however, its sensitivity is not significant, and macroscopically, integrated interpretation across the nutrient management system is possible according to the nutrient inputs into each system boundary and the nutrient uptake by plants. Nutrient management in the open-loop system has been theoretically summarized by Noordwijk (1990). This sets a tolerance factor that corresponds to a ratio between the upper and lower limit of the allowable variation range of each nutrient concentration and calculates the leaching fraction requirement for the tolerance factor. The aims of the leaching fraction application in the open-loop system are to minimize heterogeneous distribution of nutrients in the substrate and to maintain the fluctuation of the nutrient concentration in the root zone at a certain level (Noordwijk, 1990). While the nutrient control of the open-loop system is based on this theory, the actual appropriate leaching fraction is empirically determined in the experiments by applying various drainage ratios in consideration of the plant growth response (Ku and Hershey, 1991; Schon and Compton, 1997). This means that the open-loop system also has uncertainty about the change in nutrient concentration in the root zone; after all, it can be

seen that the open-loop system controls nutrient concentration by adjusting the openness of the root zone through the irrigation control without feedback on the nutrient concentration changes. Furthermore, the nutrient ratios of the open-loop system, in the absence of leaching, show rather unstable changes compared to those of the closed-loop and semi-closed-loop systems (Fig. 2.6). As described above, in open-loop soilless culture, leaching performs a homogenous distribution of nutrients in the root zone in addition to the correction of the nutrient variations. However, in open-loop systems, the leaching occurs when the nutrient solution is irrigated over the amount of evapotranspiration (Shin et al., 2014). Both nutrient correction and homogeneity are dependent on the leaching of nutrients; therefore, nutrient control in the open-loop system becomes more unstable under conditions of low or no drainage, whereas in the case of a closed-loop system, the discharge of nutrients and water to outside the system can be minimized, while the amount of drainage from the substrate is increased, which means that the homogeneous distribution of nutrients in the substrate can be performed independently of the function of nutrients correction.

Theoretical predictions for steady-state control of nutrient in the closed-loop soilless culture

It was confirmed that the nutrient changes of closed-, semi-closed-, and open-loop soilless culture systems can be macroscopically integrated in terms of nutrient input amounts. In this respect, the models of closed-, semi-closed-, and open-loop soilless culture can be simplified by the inflow and the outflow into the system boundary and by

the uptake rate in the system, which can be summarized as Eq. 2-16. And with these key parameters as the starting point of the logical process, we can reconstruct the previous perspective on the nutrient management.

$$V_{sys} \frac{dC_{sys}}{dt} = Q_{stk}C_{stk} + Q_{wtr}C_{wtr} - \frac{V_{max}C_{sys}}{K_m + C_{sys}} - Q_{out}C_{sys} \quad Eq. 2-16$$

Where Q_{stk} and Q_{wtr} are the flow rate of the stock nutrient solution and raw water flowing into the boundary of the soilless culture system, respectively and Q_{out} is the flow rate of the nutrient solution discharged outside the boundary. C_{sys} corresponds to the concentration of a nutrient in the system. C_{stk} and C_{wtr} are concentration of a nutrient in the stock solution and raw water, respectively. V_{sys} is the volume of nutrient solution in the system. V_{sys} cannot exceed the capacity of the system and the consumed water is replenished repeatedly, thus is assumed to be constant. Based on the model structure shown in Fig. 2.1, the boundary for the inflow and the outflow of the open-loop system is the substrate because there is no circulation of drainage. Therefore, Q_{wtr} and Q_{stk} without drainage reuse correspond to the irrigation rate (Q_{ir} in Eq. 2-1); and Q_{out} is the flow rate of the drained nutrient solution ($Q_{dr,n}$ in Eq. 2-1) discharged from the substrate in open-loop system. In the case of the semi-closed-loop system, the boundary of the system includes the mixing tank and the drainage tank because the drainage is partially circulated or discharged to outside the system. Therefore, Q_{stk} and Q_{wtr} correspond to the supply rate of nutrients and water to the soilless culture system, respectively; and Q_{out}

corresponds to the flow rate of the nutrient solution discharged from the drainage tank (Q_{disch} in Eq. 2-3). In the case of the closed-loop system, the boundary is same as that of the semi-closed-loop system, but Q_{out} does not occur and the steady-state solution in this case can be summarized as Eq. 2-17.

$$C_{sys} = \frac{K_m C_{stk} Q_{stk} + K_m C_{wtr} Q_{wtr}}{V_{max} - C_{stk} Q_{stk} - C_{wtr} Q_{wtr}} \quad Eq. 2-17$$

This indicates that the point of convergence for C_{sys} is determined according to the concentrations of the supplied stock solution (C_{stk}) and raw water (C_{wtr}) and their inflow rates (Q_{stk} and Q_{wtr} , respectively) and means that when the inflow rates exceed the maximum nutrient uptake rate of the plant (V_{max}), the system becomes unstable. However, in the EC-based closed-loop soilless culture system, Q_{stk} is determined based on the total equivalent concentration of nutrients in the system; as a result, the feeding rate of the total nutrients follows the uptake rate of total nutrients. Therefore, each nutrient supply rate is determined by the ratio of the nutrient in the stock solution at a level that does not continuously exceed the sum of (V_{max}) of all nutrients. However, in practice, errors may occur in the follow-up process of the total nutrient uptake rate, resulting in a fluctuation in the nutrient concentration. In this case also, its influence can be minimized and a convergent tendency in the ratio changes can be observed.

Nutrient fluctuation in closed-loop soilless culture has been defined as a problem requiring technical solution, but no specific problem definition has yet been made enough

to deduce a solid engineering design from it. Assuming that the C_{sys} is the total equivalent concentration of all nutrients in the system measured by EC and C_{sys} is controlled in steady state through EC feedback, Eq. 2-17 theoretically predicts that C_{sys} converges to a constant value. This means that adjusting the ratio of the nutrients in the stock solution supplied through Q_{stk} while performing the steady state control of the total nutrients via the EC feedback can control both the individual nutrient concentration and the ratio in steady-state. The terms including Q_{wtr} in Eq. 2-17 may contribute to the system fluctuation through the replenishment of the water consumed by the transpiration, but its influence is determined by the concentration of the nutrients in the raw water. In other words, if deionized water is used, it means that its sensitivity can be limited.

Scenario simulation for nutrient ratio control in the closed-loop soilless culture

Fig. 2.7b shows the average changes of the nutrient ratios and its standard deviations over the simulation period when performing the nutrient balance control as described in the simulation scenario. The mean values and standard deviations of K^+ , Ca^{2+} , and Mg^{2+} ratios gradually converged to the control target values and decreased as the times of nutrient ratio adjustment increase, respectively, even though the arbitrary uptake concentration was applied as a disturbance in every simulation. For each simulation, randomized transpiration rates were generated by the random walk method (Fig. 2.7a). Thus every soilless culture system simulated in this scenario has experienced different environmental conditions. Change in actual transpiration rate according to plant growth is variable depending on the environment, but they tend to increase constantly with the

growth of plants (Ta et al., 2012). In this simulation, various path of the transpiration by random walk were applied every time, within the range of maximum fluctuation during the cultivation period, which can be regarded as simulated under extreme conditions (Fig. 2.7a). Moreover, the convergence of the average nutrient ratio, even in a long-term feedback period of 12 weeks under various path of the transpiration, into the target value indicates that this system can reach an average steady state for nutrient balance. These mean that the discussion about Eq. 2-17 is also valid in the simulation under the condition with more system variables and parameters added. In a previous study, nutrient control with a two-week feedback period was conducted in an EC-based closed-loop soilless culture system (Savvas, 2002). Here, the difference between the target concentration and the current concentration at every event of nutrient analysis was applied as the feedback value. However, this method corresponds to the proportional control from the viewpoint of control engineering. The proportional control can generate a steady-state error, and results similar to this error were observed, but no discussion in this respect was conducted in that study and frequent nutrient analysis was required.

The control of the nutrient ratio simulated in this study is similar to the integral control method, in that the error is accumulated in the current input value (Golnaraghi and Kuo, 2008). This means that it is possible to access a composition of new standard nutrient solution for the steady-state of the nutrient balance change in a closed-loop soilless culture system. So far, standard nutrient solutions have been developed in consideration of each nutrient requirement ratio, chemical stability, and yield of plants through plant nutritional studies (Bautista et al., 2009; Hoagland and Arnon, 1950; Jones, 1982; Steiner, 1980).

However, considering the nonlinear nutrient uptake rate change, the standard nutrient solution and the nutrient solution to keep it in steady state may be different from each other. Standard nutrient solutions are composed of various nutrient concentrations, but most of them are reported to be contained within a certain range when converted to a ratio between nutrients (De Rijck and Schrevens, 1998). This suggests the relative stability of the nutrient ratios as a control target when considering the development process of standard nutrient solutions. Finally, through the theoretical and experimental analysis, the steady-state nutrient control technique was derived. Based on this, development of adequate techniques needs to be carried out through future research.

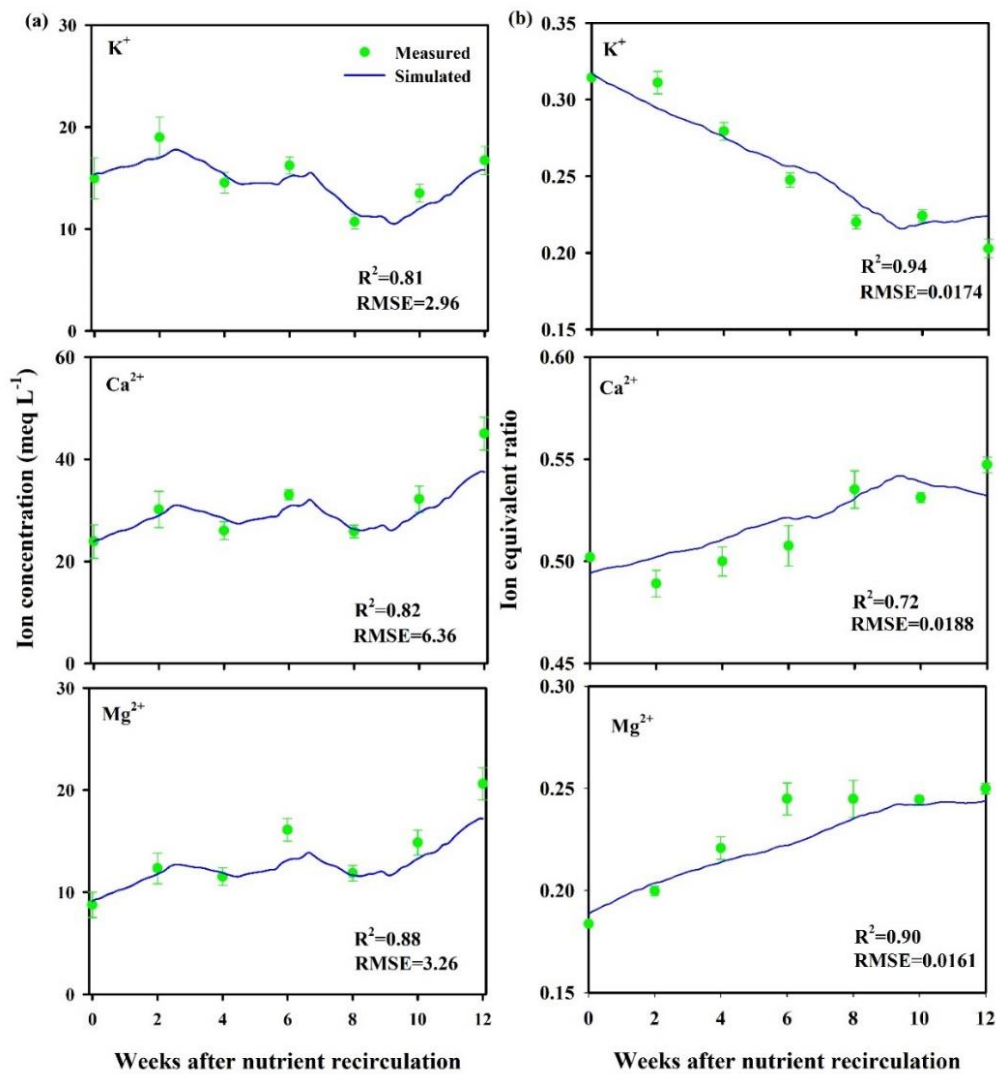


Fig. 2.3. Simulated ion equivalent concentrations (a) and ion equivalent ratios (b) in the substrates of the closed-loop soilless culture system (CL). Simulated ion equivalent concentrations of the substrates represent ion concentration in the whole layers.

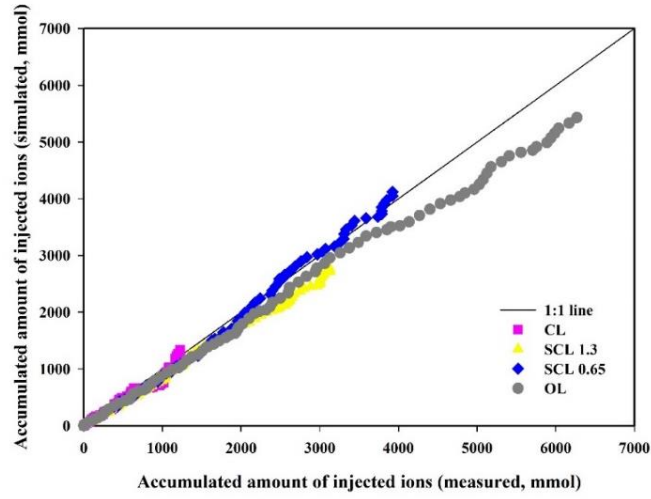


Fig. 2.4. Measured versus simulated amounts of injected total ions into the system of the closed-loop (CL), semi-closed-loop (SCL 1.3 and SCL 0.65), and open-loop (OL) soilless culture, respectively. R^2 values for the total treatments and the individual treatments of CL, SCL 1.3, SCL 0.65, and OL were 0.99, 0.95, 0.99, 0.99, and 0.99, respectively. SCL 1.3 and SCL 0.65 indicate the EC level for the dilution of the drainage by tap water in the semi-closed-loop soilless culture system.

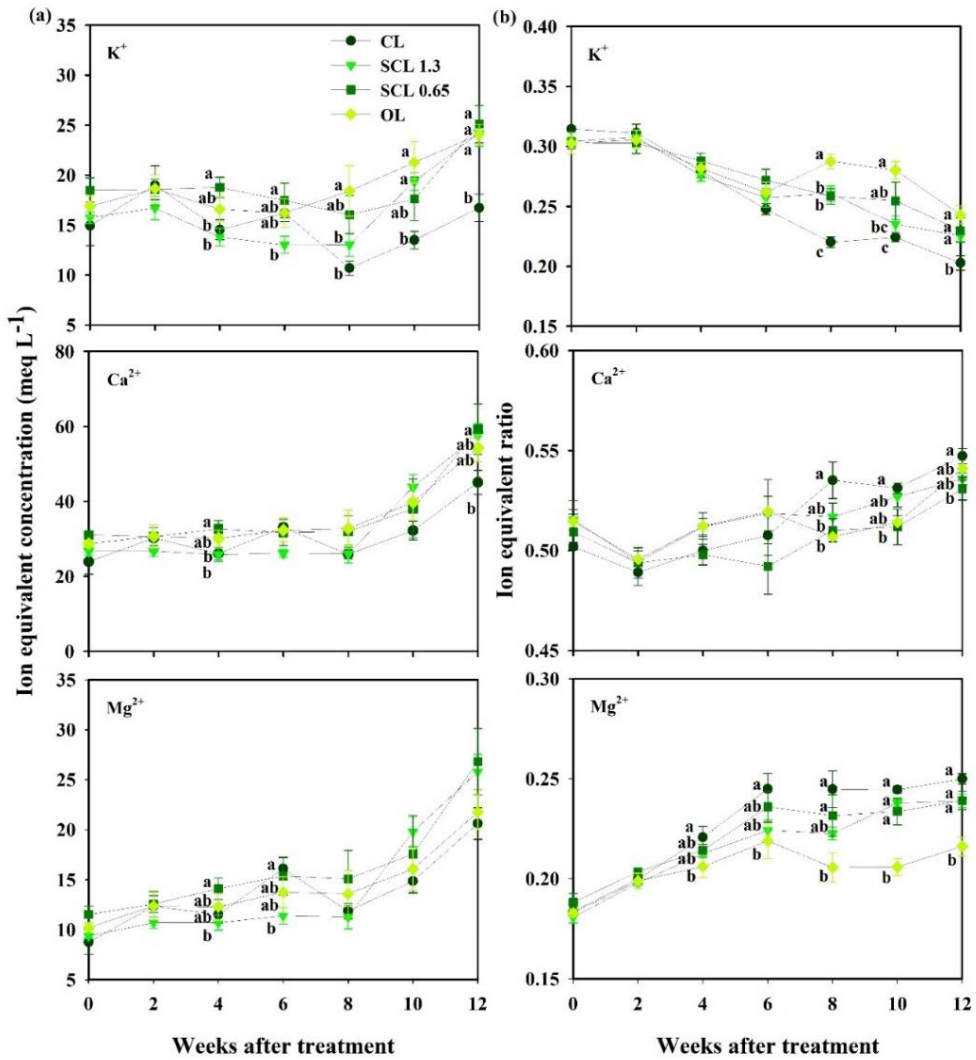


Fig. 2.5. Changes in the ion-equivalent concentration (a) and ion-equivalent ratios (b) in the substrate of the closed-loop (CL), semi-closed-loop (SCL 1.3 and SCL 0.65), and open-loop (OL) soilless culture system, respectively. SCL 1.3 and SCL 0.65 indicate the EC levels for the dilution of the drainage by tap water in the semi-closed-loop soilless culture system (Duncan's multiple range test at $P=0.05$).

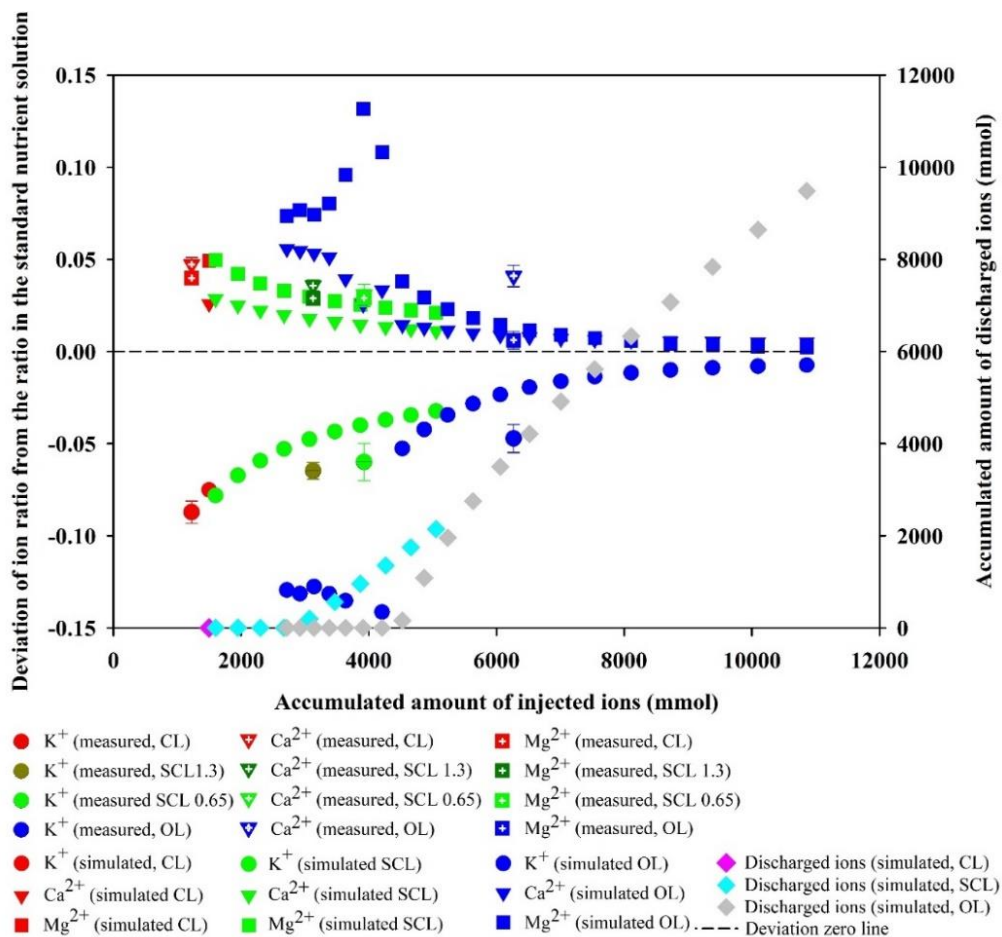


Fig. 2.6. Changes in the simulated or measured deviation of the ion ratio from the ratio in the standard nutrient solution in the substrate and the discharged nutrients ions by injected amounts of nutrients according to the soilless culture system at the end of the experiments. CL, SCL, and OL mean the closed-loop, semi-closed-loop, and open-loop soilless culture systems, respectively. SCL 1.3 and SCL 0.65 indicate the EC levels for the dilution of the drainage by tap water in the semi-closed-loop system.

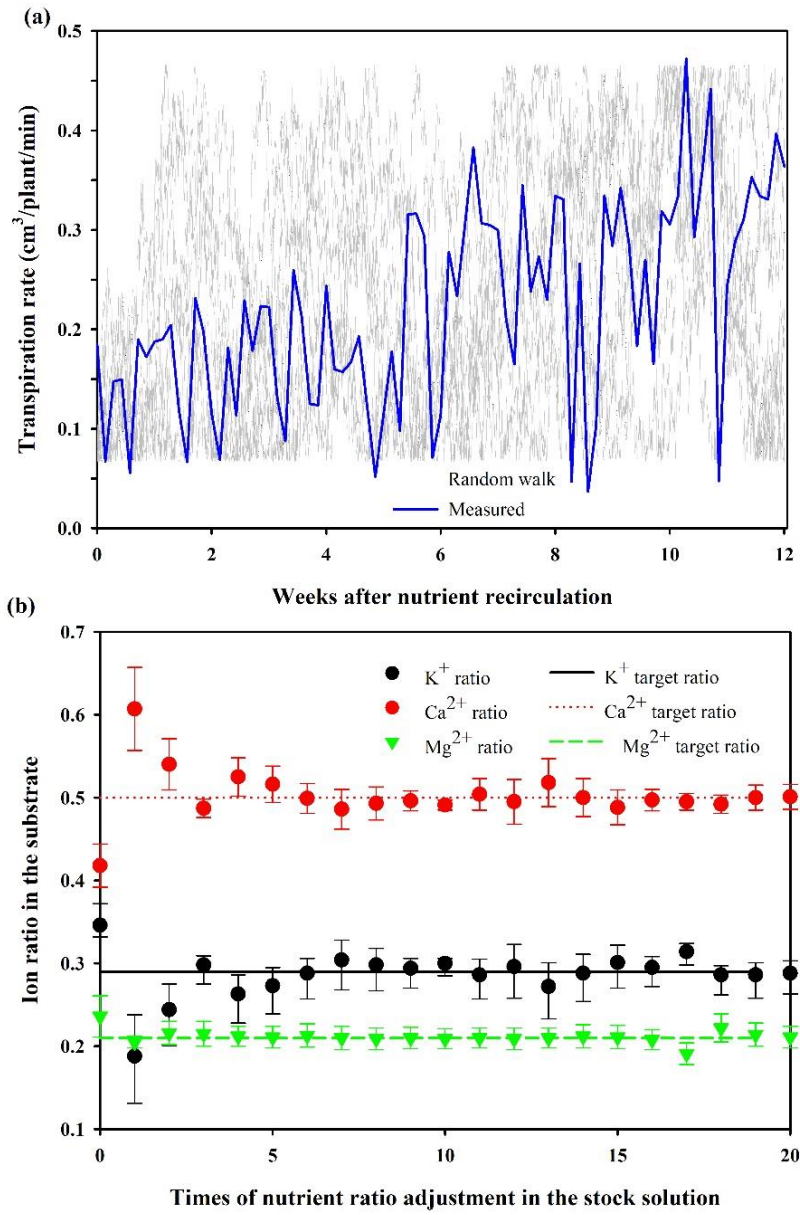


Fig. 2.7. Random walk evapotranspiration rates considering disturbance in the uptake concentrations by simulation and measured evapotranspiration rates in the experiments (a) and changes in ion ratios according to the times of adjusting the ion ratios of the stock solution under the randomized disturbance (b).

Table 2.1. Values of the parameters used for simulations of soilless culture

Parameter	Definition	Value	Unit	Source
$J_{\max} \text{ K}$	Ion flux parameter	1.89	$10^{-3} \text{ meq/plant/min}$	Estimated from the field experiment data
$J_{\max} \text{ Ca}$	Ion flux parameter	1.60	$10^{-4} \text{ meq/plant/min}$	
$J_{\max} \text{ Mg}$	Ion flux parameter	1.61	$10^{-4} \text{ meq/plant/min}$	
$K_m \text{ K}$	Ion flux parameter	9.00	$10^{-3} \text{ meq/cm}^{-3}$	
$K_m \text{ Ca}$	Ion flux parameter	2.44	$10^{-4} \text{ meq/cm}^{-3}$	
$K_m \text{ Mg}$	Ion flux parameter	1.33	$10^{-3} \text{ meq/cm}^{-3}$	
D_K	Diffusion coefficient (K^+)	1.96	$10^{-5} \text{ cm}^2 / 60^{-1} \text{ s}$	Lide (Lide, 2005)
D_{Ca}	Diffusion coefficient (Ca^{2+})	0.79	$10^{-5} \text{ cm}^2 / 60^{-1} \text{ s}$	Lide (Lide, 2005)
D_{Mg}	Diffusion coefficient (Mg^{2+})	0.71	$10^{-5} \text{ cm}^2 / 60^{-1} \text{ s}$	Lide (Lide, 2005)
W_{DAW}	Difficult available water of the substrate	0.0068	Dimensionless	Dubský M and Šrámek F (Dubský and Šrámek, 2009)
F	Field capacity of the substrate	0.74	Dimensionless	Dubský M and Šrámek F (Dubský and Šrámek, 2009)
A	Cross-sectional area of the substrate	630	cm^2	
S_{drg}	Volume of drainage tank	11700	cm^3	
S_{sub}	Volume of substrate layer n	472.5	cm^3	

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CHAPTER 3

Theoretical Analysis and Problem Definition of Nutrient Variations in Electrical Conductivity-based Closed-loop Soilless Culture Systems Using Conventional Nutrient Replenishment Method

ABSTRACT

Closed-loop soilless culture without efflux of water and nutrients is crucial for sustainable agricultural resource management. However, in the root zone of a closed-loop system, variation in nutrients can lead to instability in the nutrient management and forced discharge of nutrients and water. In an electrical conductivity (EC)–based closed-loop system, total nutrients absorbed by plants are replenished, and fluctuation in EC within a certain range around the initial value can be expected. However, this is not observed in systems using conventional nutrient-replenishment methods and the impact on nutrient management from this point of view has not been discussed. The objectives of this study were to analyze nutrient variation in a closed-loop soilless culture system based on a theoretical model and derive an alternative nutrient-replenishment method (AM). The performance of the derived AM was compared with a conventional nutrient-replenishment method (CM) through simulation analysis. A demonstration experiment using sweet peppers was then conducted to confirm whether the theoretical analysis results can be reproduced through actual cultivation. The average amounts of injected nutrients during

the experimental period (four months) in CM and AM were 16,483 and 7,722 mEq, respectively. There was no significant difference in yield of sweet peppers between the two methods. The substrate EC in the AM was maintained at $2.7 \text{ dS}\cdot\text{m}^{-1} \pm 0.5$ within the target EC value, while that in the CM gradually increased to $5.0 \text{ dS}\cdot\text{m}^{-1} \pm 1.2$. In a simulation study, results similar to the demonstration experiment were predicted. Total nutrient concentrations in the AM showed fluctuations around the target value but did not continuously deviate from the target value, while those in the CM showed a tendency to increase. Collectively, these characteristics of the AM can help minimizing nutrients and water emissions from the cultivation system.

Additional keywords: closed-loop soilless culture; electrical conductivity; nutrient solution; soilless culture; nutrient replenishment method; nutrient variation

INTRODUCTION

Closed-loop nutrient-management techniques are essential for sustainable soilless culture systems and optimal plant production, but they have not yet reached the level of technical diffusion. Nutrients in soilless culture systems are managed primarily with an open-loop nutrient supply (Bouchaaba et al., 2015; Olympios, 1999). Open-loop soilless culture systems are easier to implement, but they result in resource losses from the agricultural production system. Moreover, due to the intensive use of fertilizers, the threat posed to aquatic environments by open-loop supply practices that repeatedly discharge a certain ratio of drainage is serious enough to warrant regulation by national governments (Beerling et al., 2014; Noordwijk, 1990; Olympios, 1999; Raviv and Lieth, 2008).

Because a closed-loop soilless culture system reuses its drainage, the resulting variation in nutrient supply can have an increasingly significant effect on plant growth as the reuse period lengthens (Kläring, 2001; Moon et al., 2018; Noordwijk, 1990; Zekki et al., 1996). It is therefore difficult to intuitively explain or interpret nutrient-variation management techniques, unlike an open-loop system. Appropriate application of techniques and an explanation of the effects of their application require a theoretical model and a precisely defined problem (Eggert, 2005).

In both closed-loop and open-loop soilless culture systems, the electrical conductivity (EC) of the nutrient solution in the mixing tank is adjusted to a target value before the solution is applied to the plant (Putra and Yuliando, 2015; Savvas and Manos, 1999; Zekki et al., 1996). However, unlike an open-loop system, the mixing ratio of tap water to stock solution in a closed-loop system is adjusted by considering the change in nutrient

concentration due to the inflow of drainage (Savvas and Manos, 1999). Alternatively, in simple systems, a premixed standard nutrient solution of a certain EC is supplied based on the difference between the initial and current water levels in the circulation tank, which simultaneously performs drainage collection and nutrient-solution feeding (Massa et al., 2011; Signore et al., 2016).

In an EC-based closed-loop soilless culture system, supply of stock solution or standard nutrient solution and tap water is intended to replenish nutrients and water consumed in the system (Savvas and Manos, 1999). For a single system in which the plants are grown directly in a nutrient solution container, the nutrients and water consumed due to absorption of plants in the system can be estimated almost exactly (Anpo et al., 2018). However, errors may occur in systems in which the root zone and nutrient supply are separate from drainage collection. Both elements are widely used in commercial farming conditions,

Considering the functional objective of nutrient and water replenishment in a closed-loop soilless culture system, relatively stable fluctuations within a certain range around the initial EC value should be observed. However, in actual closed-loop soilless culture system, the EC variation is similar to the variation of an open-loop system (Hao and Papadopoulos, 2002; Massa et al., 2011; Signore et al., 2016). In addition, the effects of these fluctuations are linked to forced discharge of recirculated nutrient solution to outside of the system (Massa et al., 2011; Signore et al., 2016).

The problems associated with variations in nutrient concentration or EC observed in soilless culture systems are presumed to be inevitable, as the nutrient uptake concentration

is affected by the environment (Noordwijk, 1990; Signore et al., 2016). The experimental results are interpreted depending on the responses of the system according to treatment application (Hao and Papadopoulos, 2002; Incrocci et al., 2006; Rouphael et al., 2016; Signore et al., 2016; Zekki et al., 1996), and these have proven difficult to interpret in an integrated way. As a result, technical approaches to managing nutrient variation and the design of experiments are limited. To block nutrient emissions from a soilless culture system, nutrient reuse practices must be standardized, a task that requires precise problem definition based on variations of nutrient concentrations or EC.

The objectives of this study were to analyze the cause of EC variation in closed-loop soilless culture systems based on a theoretical model, to derive an alternative nutrient-replenishment method for management of nutrient fluctuation, and to evaluate the performance of the method through theoretical and experimental analyses.

MATERIALS AND METHODS

Soilless culture system model

The model used in this study simulated nutrient changes in an automated soilless culture nutrient solution mixing system consisting of a mixing tank, a medium, and a drain tank (Fig. 3.1). The basic structures of the soilless culture system and plant growth models were constructed by referring to the nutrient solution transport model in a substrate condition (Raviv and Lieth, 2008; Silberbush et al., 2005; Silberbush and Ben-Asher, 2001; Snape et al., 2008). The measured value of incident radiation intensity was used as an input variable in the simulation.

Water and nutrients transport in substrate

According to standard practices for automated irrigation of a soilless culture system, the mixing process for a nutrient solution is initiated in the mixing tank, and the nutrient solution is supplied to the substrate after mixing. The target nutrients for the simulation were selected as macronutrient cations (K^+ , Ca^{2+} , and Mg^{2+})

$$\frac{dV_n}{dt} = Q_{n-1} - Q_n - T_n \quad Eq. 3-1$$

where V_n is the volume of water in the substrate layer n . Q_{n-1} and Q_n are the flow rates of the drain from the $n - 1$ layer and to the $n + 1$ layer, respectively, and T_n represents the evapotranspiration rate of the substrate layer n . In the first substrate layer

($n = 1$), Q_{n-1} is the irrigation flow rate to the top of the substrate, Q_n is the difference between the flow rate of the drain from the substrate layer $n - 1$ and the evapotranspiration rate in the substrate layer n ($Q_{n-1} - T_n$) or the difference between the irrigation rate and the evapotranspiration rate in the first substrate layer ($Q_0 - T_1$) (Shin et al., 2014). The field capacity (F) and difficult available water (W_{DAW}) respectively restrict Q_n and T_n . The flow for Q_n occurs only when $V_n > S_{sub}F$, and T_n flows only when $V_n > S_{sub}W_{DAW}$.

The flow of nutrients in the medium is generated by the flow rate of water.

$$V_n \frac{dC_n^I}{dt} = Q_{n-1} \cdot C_{n-1}^I - Q_n \cdot C_n^I - P_{RSA} \cdot J_n^I \quad Eq. 3-2$$

where C is the equivalent concentration of nutrient, superscript I is the type of ions (K^+ , Ca^{2+} , Mg^{2+}), J_n^I is the uptake rate of nutrients, and P_{RSA} is the specific root surface area, which is described as root length density and specific root surface area.

Plant variables and growth parameters

In this simulation, evapotranspiration and nutrient uptake rates were applied as plant variables in the substrate. In general, the plant parameters relate to change in evapotranspiration and nutrient uptake rates with plant growth. The relationship between solar radiation and evapotranspiration is adjusted by the leaf area index (Baille et al., 1994). The parameters related to nutrient uptake rate are derived from the characteristics of the

plant ion transporters and are modeled as increasing with growth of root surface area (Bassirirad, 2000).

Leaf area index

The Boltzmann sigmoid equation was used to apply changes in leaf area index to the evapotranspiration rate:

$$P_{LAI,t} = \frac{a}{\left[1 + e^{\frac{x_0 - t}{b}}\right]} \quad Eq. 3-3$$

where a , b and x_0 are the constants, and t is time.

Evapotranspiration

The evapotranspiration rate was modeled using the equation derived from Penman–Monteith equation:

$$T_n = a[1 - e^{-k_{LAI}P_{LAI}}] \frac{R}{\lambda} + b \quad Eq. 3-4$$

where T_n is the evapotranspiration rate, R is the incident radiation intensity, λ is the latent heat of vaporization, k is the light extinction coefficient, a and b are regression parameters, and LAI is leaf area index.

Root length density and specific root surface area

Root length density was used to calculate specific root surface area and modeled using a logistic function of time (Barber, 1995; Silberbush and Ben-Asher, 2001):

$$P_{len,t} = \frac{RLD_{max}}{1 + K_1 e^{-k_1 t}} \quad Eq. 3-5$$

where RLD_{max} is the maximal root length density, and K_1 and k_1 are coefficients.

Specific root surface area was calculated as follows:

$$P_{RSA,t} = 2\pi r_0 P_{len,t} \quad Eq. 3-6$$

where r_0 is mean root radius. Root length density was set to start at the top layer of the substrate and be sequentially assigned to the subsequent layer as the value increased. The allocation of root length density for each layer was calculated by dividing RLD_{max} by the total number of layers.

Nutrients uptake

The nutrient uptake rate of the plant in the substrate was simulated as a function of Michaelis–Menten:

$$J_n^I = \frac{J_{max}^I (C_n^I - C_{min}^I)}{K_m^I + (C_n^I - C_{min}^I)} \quad Eq. 3-7$$

where J_{max}^I is the maximum absorption rate of nutrient I , K_m^I is the Michaelis–Menten constant, and C_{min}^I is the minimal concentration at which $J_n^I = 0$.

Conventional mixing control of nutrient solution in the simulation

The mixing process for stock solution, tap water, and drainage under the automated closed-loop soilless culture system is performed in the mixing tank (Putra and Yuliando, 2015; Savvas and Manos, 1999). When the system receives an irrigation command, the entire volume of drainage is diluted with tap water within the range of the irrigation volume, and the stock solution is added to the target EC. This is the approach described in the Introduction, where the open-loop system adjusts the EC in the mixing tank to a fixed target.

However, because drainage is included in the automated mixing process in closed-loop soilless culture systems, the equation needs to solve for target EC with mixing stock solution, drainage, and water (Savvas and Manos, 1999). The nutrient solution mixing process occurs intermittently according to irrigation interval, and the basic equation for conventional nutrient replenishment can be summarized based on the dilution equation:

$$V_T C_T = V_D C_D + V_W C_W + V_S C_S \quad \text{Eq. 3-8}$$

where V_T is the target irrigation volume per event, C_T is the target total equivalent concentration, V_D is drainage volume, C_D is total equivalent concentration in drainage,

V_W is the amount of tap water input to the mixing tank, V_S is the amount of stock solution input to the mixing tank, and C_S is the concentration of the stock solution. Eq. 3-8 can be summarized as Eq. 3-10 by substituting Eq. 3-9 for V_W .

$$V_W = V_T - V_D - V_S \quad \text{Eq. 3-9}$$

$$V_S = \frac{C_T V_T - C_W V_T + C_W V_D - C_D V_D}{C_S - C_W} \quad \text{Eq. 3-10}$$

The amount of stock solution input to the mixing tank is calculated through this process, and when the irrigation control command is generated during the simulation, the mixing calculation begins based on the volume of drainage stored in the drainage tank at that moment. If the calculated value of the Eq. 3-10 is less than 0, dilution with tap water cannot be adjusted to the target concentration within the range of the irrigation amount. In this case, the amount of tap water required to dilute the drainage to the target C_T is calculated, and then the ratio between drainage and calculated tap water is multiplied by target irrigation amount (V_T).

When the doses of V_D , V_W , and V_S are determined through the abovementioned calculation, a flow rate is generated until the corresponding amount is transferred to the mixing tank (V_M) according to the flow rates of drainage (Q_{drg}), tap water (Q_{wtr}), and stock solution (Q_{stk}), respectively.

In the simulation, irrigation was controlled by a radiation integral method, which is conventionally used in automated irrigation control (Shin and Son, 2016). Nutrient solutions measuring 140 mL per plant in the mixing tank were supplied whenever the accumulated radiation reached 100 J m^{-2} . The data for incident radiation intensity (R) were collected by pyranometer (SP-110-L10, Apogee Instruments, Logan, Utah, USA) in a Venlo-type greenhouse at the experimental farm of Seoul National University (Suwon, Korea, Lat. 37.3° N , long. 127.0° E) from September 10, 2011 to March 9, 2012 (Fig. A.1).

Experimental analysis

Cultivation conditions

Three sweet pepper (*Capsicum annuum* L. “Derby”) plants were grown in a rockwool slab, and seven slabs were used per row. In this study, four cultivation lines were installed in the greenhouse, each of which was an independent closed-loop soilless culture system with a mixing tank, drainage tank, and stock solutions. The stock solution was prepared based on the PBG nutrient solution of the Netherlands. In the greenhouse, daytime temperature was maintained at $25\text{--}35^\circ \text{ C}$ and nighttime temperature at $17\text{--}22^\circ \text{ C}$. Solar radiation-based irrigation control was applied; when the cumulative radiation reached $100 \text{ J} \cdot \text{cm}^{-2}$, 150 mL of the nutrient solution was supplied to each plant. However, the irrigation amounts were adjusted according to meteorological conditions to maintain a drainage ratio of approximately 30%. The composition of nutrient solution was $14.17 \text{ mEq} \cdot \text{L}^{-1}$ of NO_3^- , $1.14 \text{ mEq} \cdot \text{L}^{-1}$ of H_2PO_4^- , $5.92 \text{ mEq} \cdot \text{L}^{-1}$ of K^+ , $8.85 \text{ mEq} \cdot \text{L}^{-1}$ of Ca^{2+} , $3.17 \text{ mEq} \cdot \text{L}^{-1}$ of Mg^{2+} , and $3.20 \text{ mEq} \cdot \text{L}^{-1}$ of SO_4^{2-} as macro-elements; and $0.038 \text{ mEq} \cdot \text{L}^{-1}$ of Fe^{2+} , $0.020 \text{ mEq} \cdot \text{L}^{-1}$

¹ of Zn^{2+} , 0.003 $\text{mEq}\cdot\text{L}^{-1}$ of Cu^{2+} , 0.021 $\text{mEq}\cdot\text{L}^{-1}$ of Mn^{2+} , and 0.001 $\text{mEq}\cdot\text{L}^{-1}$ of MoO_4^{2-} as micro-elements. After an irrigation event, the drainage solution was returned to the drainage tank (11.7 L). The EC and pH of tap water were 0.17 $\text{dS}\cdot\text{m}^{-1}$ and 7.11, respectively, and contained 0.21 $\text{mEq}\cdot\text{L}^{-1}$ of Na^+ , 0.29 $\text{mEq}\cdot\text{L}^{-1}$ of Cl^- , 0.04 $\text{mEq}\cdot\text{L}^{-1}$ of K^+ , 0.71 $\text{mEq}\cdot\text{L}^{-1}$ of Ca^{2+} , 0.21 $\text{mEq}\cdot\text{L}^{-1}$ of Mg^{2+} , 0.19 $\text{mEq}\cdot\text{L}^{-1}$ of SO_4^{2-} , 0.39 $\text{mEq}\cdot\text{L}^{-1}$ of NO_3^- , and 0.04 $\text{mEq}\cdot\text{L}^{-1}$ of PO_4^{3-} .

Measurement of fruit yield and, analyses of nutrient content in leaves and root zone

The total yield and average fruit weight during the experimental period were measured. The proportion of blossom-end rot (BER) fruits on a sweet pepper plant was measured. At the end of the experiment, 18 leaves (including petiole) from the middle to the top nodes of a sweet pepper were randomly collected from each treatment. Leaves were washed in tap water and dried for 48 hours at 70°C in an oven. The dried leaves were ground, and 0.5 g of each ground sample was digested using concentrated nitric acid. Next, 1 mL of concentrated perchloric acid was added to maintain a set solution temperature of 180°C, and the digestion process was accelerated on a hot plate at 90°C for approximately one hour, until a clear-colored solution was obtained. After digestion, the tube was cooled, filled with 25 mL deionized water, and the total contents of K^+ , Ca^{2+} , and Mg^{2+} in the leaves were determined with an ICP-730ES spectrometer (ICP-730ES, Varian, Mulgrave, Australia). To determine the changes of nutrients in the root zone, samples of nutrient solution in the rockwool slabs were extracted using a syringe. The collection points of the nutrient solution in the rockwool slab were randomly selected to ensure representative

samples of the overall concentration in the root zone. Five 10 mL samples of a root zone nutrient solution were collected for each extraction, for a final volume of 50 mL sample. Four 50 mL samples per treatment were collected every week. SAS (version 9.2, SAS Institute, USA) was used for statistical analysis.

Nutrient replenishment method

A conventional nutrient-replenishment method (CM) and an alternative nutrient-replenishment method (AM) derived from the theoretical analysis in this study were performed in the mixing tank with two applied nutrient solution mixing modules. In the CM, when the system received an irrigation command, the entire drainage volume was diluted with tap water within the range of irrigation volume, and the stock solution was added to match the fixed target EC (Savvas and Manos, 1999). In the AM, the additional volume of the stock solution was determined by the equation derived from the simulation study at every irrigation event (Eq. 3-14).

Nutrient solution mixing module and data collection

The ECs of the nutrient solutions in the mixing tank and drainage tank were measured by EC sensors (SCF-01A, DIK, Korea). Light intensity in the greenhouse was measured with a pyranometer (SP-110, Apogee, United States) and used for input data for solar radiation-based irrigation control. Data were measured every 10 seconds from October 15 to December 31, 2014. Mean values for every hour were used. A CR1000 datalogger (Campbell Scientific, USA) was used to measure and control the drainage and nutrient

mixing process. Water levels of the stock solution tanks and the drainage tanks were monitored by ultrasonic sensors (UHA-300, Unics, Korea) and used to estimate the stored volume changes of stock and drainage solutions. ECs in the root zone were measured at intervals of two to five days using a multimeter (Multi 3420 SET C, WTW, Germany).

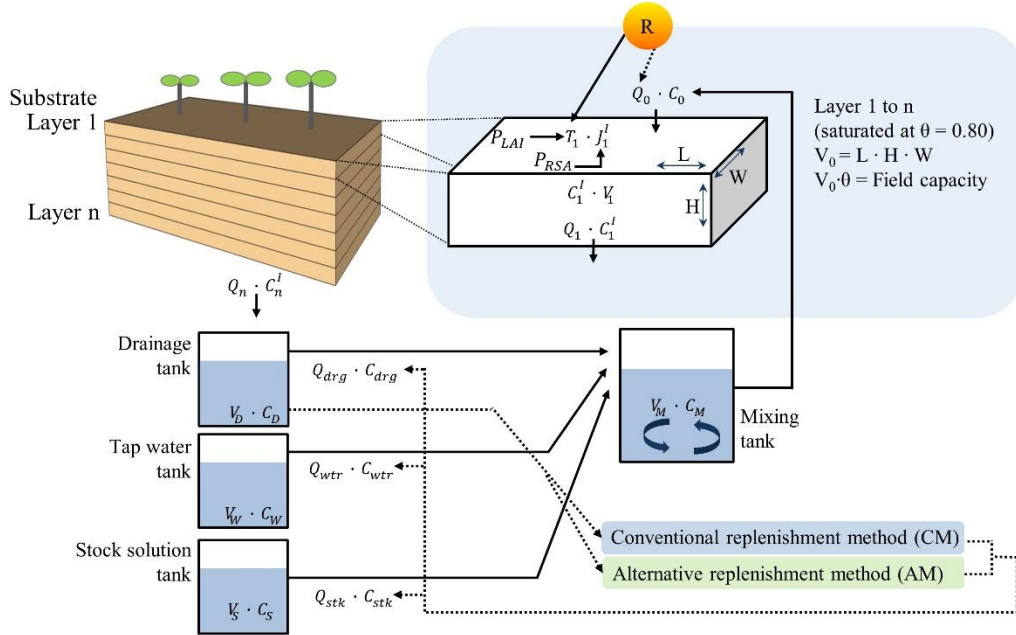


Fig. 3.1. Schematic description of a closed-loop soilless culture system in a simulated condition. Solid lines indicate water and nutrient flow, and dotted lines indicate data flow for nutrient solution mixing and irrigation control. CM and AM mean conventional and alternative nutrient-replenishment methods, respectively.

RESULTS AND DISCUSSION

Theoretical Analyses

Analyses of conventional nutrient replenishment

Total ion concentration in the substrate and total ions in the soilless culture system were analyzed (Fig. 3.2a). The total concentration of nutrients in the system gradually increased, but after approximately 60 days, repeated fluctuations within a certain range were observed, and no increased tendency was detected. Irregular fluctuations with an increasing tendency in total nutrient concentrations relative to initial values have been reported in most closed-loop, semi-closed-loop, and open-loop soilless culture systems (Hao and Papadopoulos, 2002; Lee et al., 2017; Massa et al., 2011; Moon et al., 2018; Signore et al., 2016).

Fig. 3.2b shows total nutrient amounts in the simulated closed-loop soilless culture system. The increase in the system suggests that the CM consistently failed to replenish the actual nutrient uptake.

Previous studies of closed-loop soilless culture systems have attributed irregular fluctuations in total nutrient concentrations to the difference between the concentration of irrigated nutrient solution and nutrient uptake concentration, which dynamically changes depending on environmental conditions. Furthermore, most studies of nutrient changes in closed-loop systems have been carried out based on the premise that nutrient variations are the result of the changing dynamics of uptake concentration (Carmassi et al., 2007, 2005; Hao and Papadopoulos, 2002; Noordwijk, 1990; Savvas, 2002; Signore et al., 2016).

Problem definition in a closed-loop soilless culture system

From a whole-system perspective, water consumed by evapotranspiration can be eventually detected through changes in the stored volume of nutrient solution. It is therefore technically advantageous to maintain a certain water level in the system. Furthermore, even though a cultivation system is divided into sub-systems such as root zone, drainage tank, and mixing tank, the approximate concentration of total nutrients in the system can be fed back via EC measurement. This means that, if stock solution input (V_S in Eq. 3-10) is adjusted according to EC measurement, total nutrient concentration in a closed-loop soilless culture system can be maintained at an average steady state.

However, almost all closed-loop nutrient management research to date has been based on the problem definition and interpretation domain of an open-loop soilless culture system. This means that the system for determining the influx of nutrients and water into the system has been designed in the same way as the open-loop. The effects of nutrient and water replenishment in a closed-loop system divided into sub-systems such as root zone, drainage tank, and mixing tank (a simple system) cannot be properly interpreted the same way they would be in a cultivation system with only a single root-zone container. Starting from a single system that controls only the liquid container comprising the root zone, the problem is defined as follows:

$$V_0C_0 = V_1C_1 + V_U C_U \quad \text{Eq. 3-11}$$

where V_0 is initial volume of nutrient solution in the plant root container, C_0 is initial total nutrient concentration in the plant root container, V_1 is current volume of nutrient solution, C_1 is current concentration of total nutrient, V_U is amount of water absorbed by the plant, and C_U is average total nutrient uptake concentration.

However, when a closed-loop soilless culture system is divided into substrate, drainage, and mixing tank, V_0 and C_0 in Eq. 3-11 become the irrigation volume in the mixing tank and the target total nutrient concentration, respectively. That is, replenishment of nutrients and water is not calculated for all nutrients in the system but only for those in the mixing tank. To theoretically remove the errors in calculation associated with nutrients and water replenishment, the system-wide nutrients and water need to be considered as follows:

$$V_0 C_0 = V_{drg} C_{drg} + V_{sub} C_{sub} + V_{mix} C_{mix} + V_U C_U \quad Eq. 3-12$$

where V_0 is the initial volume of water in the system; C_0 is the initial total concentration of the system; V_{drg} , V_{sub} , and V_{mix} are the volumes of water stored in the drainage tank, substrate, and mixing tank, respectively; and C_{drg} , C_{sub} , and C_{mix} are the total nutrient concentrations in the drainage tank, substrate, and mixing tank. Summarizing the equation with respect to amount of nutrient uptake ($V_U C_U$), it is possible to calculate the amount of total nutrients to be added to maintain an average steady state in the system.

Derivation of possible solution

To calculate the amount of nutrient replenishment in the actual system, Eq. 3-12 needs to be rewritten, with the amount of nutrient uptake ($V_U C_U$) substituted for the input volume of stock solution (V_{stk}) for V_U and stock solution concentration for C_U . V_{stk} can then be expressed as follows:

$$V_{stk} = \frac{C_0 V_0 - C_{drg} V_{drg} - C_{mix} V_{mix} - C_{sub} V_{sub}}{C_{stk}} \quad Eq. 3-13$$

The variables in Eq. 3-13 are available in the simulation, and a comparison of the CM and the AM is presented in Fig. 3.3. For total nutrient concentration, the average steady-state error was observed in the system using CM, while repeated fluctuations were observed for AM, although the concentration did not continuously deviate significantly from the target value (Fig. 3.3a). The amount of total nutrients in the system also increased in the CM; in the AM, it stayed near the initial value without any obvious increasing or decreasing tendency (Fig. 3.3b).

Precise measurements for the variables in Eq. 3-13 in a real cultivation system have technical limitations. In particular, the amounts of total nutrients C_{sub} and V_{sub} in the substrate are difficult to estimate. In a soilless culture system, the field capacity (F) of a substrate corresponds to the parameters of the system, and the volume of water cannot exceed the volume of the root zone multiplied by field capacity (F_{est}). The EC of the drainage (C_{drg}) can be indicative of a change in concentration of substrate. Considering this, we can modify the Eq. 3-13 as follows:

$$V_{stk} = \frac{C_0V_0 - C_{drg}V_{drg} - C_{mix}V_{mix} - C_{drg}F_{est}}{C_{stk}} \quad Eq. 3-14$$

When C_{drg} and F_{est} are substituted for C_{sub} and V_{sub} , respectively, errors may occur. However, in this case, total ion concentration fluctuated around the initial concentration (Fig. 3.4a). When F_{est} was adjusted, the same tendency was observed, but the fluctuation was attenuated (Fig. 3.4b).

For these analyses to be implemented, Eq. 3-14 must be reviewed from the perspective of control engineering. In Eq. 3-14, all but C_{drg} can be viewed as parameters. That is, the process of calculating the difference between C_0V_0 and the product of the parameters and C_{drg} is performed in every mixing process. From this point of view, Eq. 3-14 is structurally similar to proportional control:

$$u(t) = K_p e(t) \quad Eq. 3-15$$

where u is controller output, t is time, K_p is proportional gain, and e is instantaneous process error at time t (Golnaraghi and Kuo, 2008). In the existing problem definition, the EC variation in the closed-loop soilless culture system was derived from the dynamic change of the uptake concentration; thus, there were restrictions on active control and interpretation. However, a series of analysis steps leading to Eq. 3-15 makes it possible to

convert EC control in the closed-loop soilless culture system to the problem of proper gain search through arbitrary adjustment of system parameters.

Experimental analysis

The AM showed stable changes in the root zone EC control against the CM (Fig. 3.5). While the root zone EC in the AM was maintained near the initial value of the system, an increasing tendency in stored drainage volume in the drainage tank was not observed (Fig. 3.6). The average level of stored drainage level in the CM was higher than in the AM, and the range of variation was relatively wider (Fig. 3.6). As described in Materials and Methods, the mixing ratio of drainage, water, and stock solution in the conventional nutrient solution mixing process depends on target EC for the irrigation solution. However, this aspect could generate significant fluctuations in the stored volume of drainage. The influence of CM on the stored volume of drainage is discussed further in Supporting information section 1 in Appendix. No increasing or decreasing trend in EC or stored drainage volume can be inferred over the entire experimental period in the closed-loop system, meaning that total nutrient input to the system adequately followed total nutrient uptake by the plant. In the CM, the EC of the root zone was relatively higher, and gradual increase was observed. The EC in the root zone can eventually be reflected in the EC of the drainage. A high EC value in a closed-loop soilless culture system where concentration control of the recycled nutrient solution is carried out can lead to an increase in volume of stored drainage solution and subsequently to discharge of drainage when it exceeds system capacity (Massa et al., 2011; Signore et al., 2016). This can be a factor in system instability.

The EC changes in the root zone of the AM applied system indicate a normal effect of the proportional gain adjustment, as in the theoretical analysis in this study.

The cumulative amount of nutrients supplied to the system using the AM increased at a low rate in comparison with the CM, and the final amount of supplied nutrients was also lower than that of the CM (Fig. 3.7). The AM appeared to work normally, and a reduction in fertilizer input compared with the CM was also observed in the supplemental result of the simulation analysis (Fig. A.4; Supporting information section 2 in Appendix). In addition, measurement of cumulative amount of nutrients in a state with no overall increases in EC and stored drainage volume were not observed indicates that the system can detect the total nutrient requirement of a plant. This measure could be used as an index for plant nutritional status, one that is not provided in the CM.

In the case of stock solution input volume change, it was confirmed that the input amount of the AM was relatively evenly distributed during the cultivation period (Fig. 3.8b). On the other hand, in the case of the CM, a concentrated period of nutrient solution injection occurred, and relatively long periods during which the input of stock solution was blocked were observed (Fig. 3.8a). The irregular feeding rate of the stock solution could be an adverse factor in nutrient-balance control when nutrient correction in the system is performed by input of stock or standard nutrient solution (Massa et al., 2011; Savvas, 2002; Signore et al., 2016). This was also observed in the supplemental results of the simulation analysis (Fig. A.3; Supporting information section 2 in Appendix).

In the CM, overall tendencies of increasing Ca^{2+} and Mg^{2+} and decreasing K^{+} were observed (Fig. 3.9). In the AM, Ca^{2+} and Mg^{2+} concentrations were stable at a level

relatively close to the initial value, but K^+ values showed a rapid decline and then fluctuated at a low concentration (Fig. 3.9). For CM, variations in nutrient concentrations similar to those reported in previous studies were observed (Hao and Papadopoulos, 2002; Signore et al., 2016; Zekki et al., 1996). Previous research on closed-loop soilless culture systems has determined that nutrient variations are a result of dynamic changes in nutrient uptake concentrations, and following those changes is challenging. (Carmassi et al., 2007; Noordwijk, 1990; Savvas et al., 2007; Signore et al., 2016). However, Fig. 3.9 indicates that a more deterministic change occurred in the system when nutrient replenishment was synchronized with total nutrient uptake through the AM system. The cumulative standard deviations of the AM were maintained at a lower level than those of the CM during the entire experimental period, and gradually decreasing tendencies were observed in K^+ and Mg^{2+} for the AM (Fig. 3.10). This means that the changes in nutrient concentration in the AM applied system were maintained close to the average concentration values during the experimental period compared with the CM. Considering the nutrient variations of the AM system itself, there may be a limit to defining it as steady state in the strict sense. However, in the actual cultivation conditions in this experiment, input of nutrients and water into the root zone by irrigation occurs intermittently, and the variation in the section where no input occurs cannot be controlled until the next input event. Furthermore, the frequency of changes of such input can affect system fluctuations (Ta et al., 2012; Xu et al., 2004), and the AM applied system is also under this influence. Considering these constraints and the CM changes, it can be assumed that the AM entered an average steady state that fluctuated within a certain range. The nutrient concentration control in the soilless culture system can

therefore be seen as shifting the fluctuation range of the average steady state to the target range through a compositional change in the stock nutrient solution.

However, because the K^+ concentration of the AM was maintained at a very low level in this study, the impacts on sweet pepper productivity need to be considered. Total sweet pepper yields during the experiment were compared (Fig. 3.11). The average total yield was 827.5 g per plant (standard deviation [SD] ± 106.5) in the CM and 838.8 g per plant (± 109.8) in the AM, and significant differences were not observed. The average fruit weights were 133.7 g (± 35.2) and 137.8 g (± 38.6) for the CM and AM, respectively, but no significant effect was observed. In the case of blossom-end rot, the mean value was low in the AM but not by a significant difference (Fig. 3.12). This is considered to be due to the difference in concentration of the root zone when considering the characteristics of sweet pepper responses to root zone nutrient concentration (Tadesse et al., 1999).

When comparing the changes in nutrient ratio in the root zone during the experiment, the AM showed a tendency to accumulate calcium (Fig. 3.13), but it was not in the range of physiological limitations of Steiner's standard (Steiner, 1980). Leaf analysis confirmed that absorption selectivity is maintained by achieving the ratio range of standard nutrient solutions, unlike the ratio of nutrients in the root zone nutrient solution (Fig. 3.13). In the AM, the concentration of K^+ was maintained at a low level, but the supply interval of the stock solution was relatively uniformly distributed, resulting in a periodic supply. That could correspond to the prevention effect of nutrient deficiency through the constant feeding rate of nutrients even at lower concentrations (Ingestad, 1982).

The effects of synchronized total nutrient supply on total nutrient uptake by the alternative nutrient-replenishment method (AM) were confirmed and compared with those of the conventional nutrient-replenishment method (CM). In the AM, electrical conductivity (EC) was maintained close to the initial value, and the use of fertilizers was reduced by about 45% without significant yield losses compared with the CM. This could mean that a closed-loop soilless culture system, showing complicated nutrient variations, can be stably controlled. Through this study, the problem of EC variation in closed-loop soilless cultures was theoretically analyzed. In addition, more advanced control techniques could be applied based on the problem definition provided by this study.

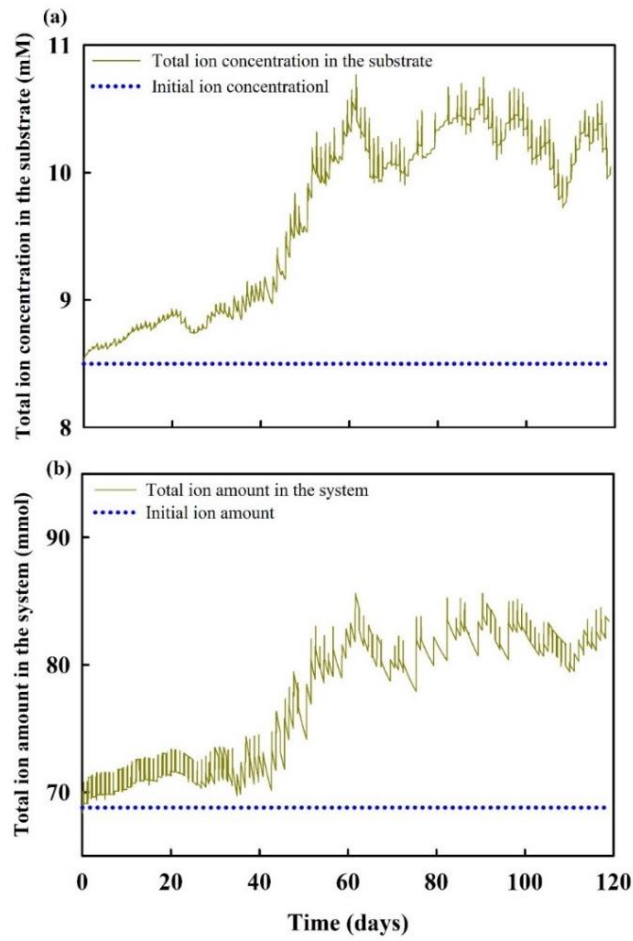


Fig. 3.2. Changes in total ion concentration in the substrate (a) and total ion amount in the system (b) in a closed-loop soilless culture system using the conventional nutrient-replenishment method.

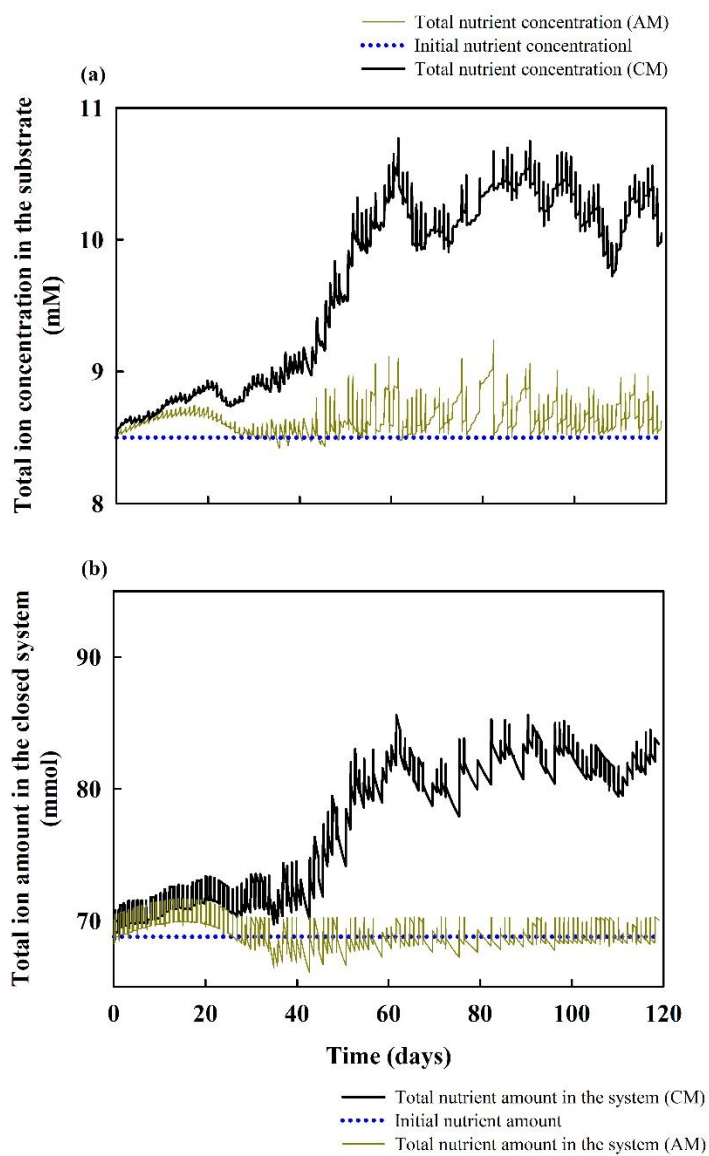


Fig. 3.3. Changes in total ion concentration in the substrate (a) and total ions in the system (b) in the closed-loop soilless culture system using conventional and alternative nutrient-replenishment methods.

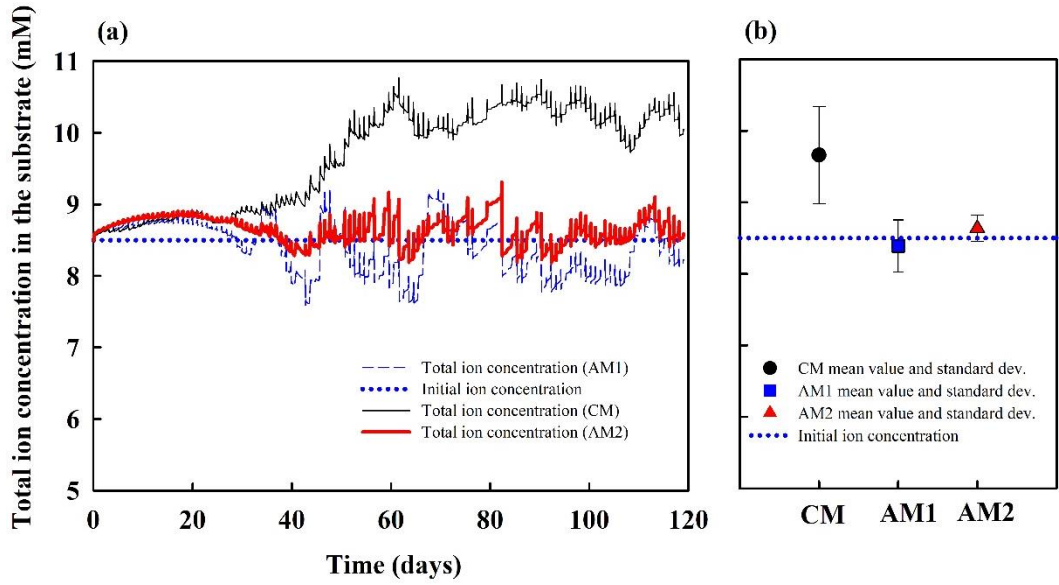


Fig. 3.4. Changes in total ion concentration in the substrate according to the nutrient-replenishment method (a) and mean and standard deviation of total ion concentration in the substrate according to the nutrient-replenishment method (b); CM is conventional nutrient-replenishment method, AM1 is alternative nutrient-replenishment method with estimated field capacity (F_{est}) in Eq. 3-14, and AM2 is alternative nutrient-replenishment method with the estimated field capacity in Eq. 3-14.

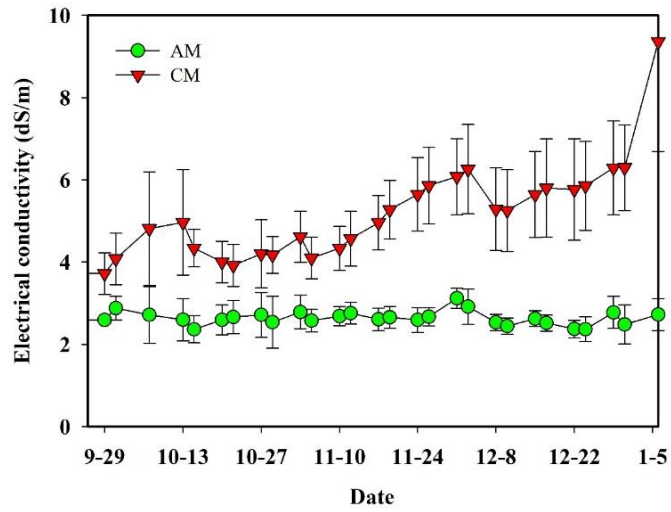


Fig. 3.5. Comparison of electrical conductivity in the root zone of a closed-loop soilless culture system during the experimental period between conventional (CM) and alternative (AM) nutrient-replenishment methods.

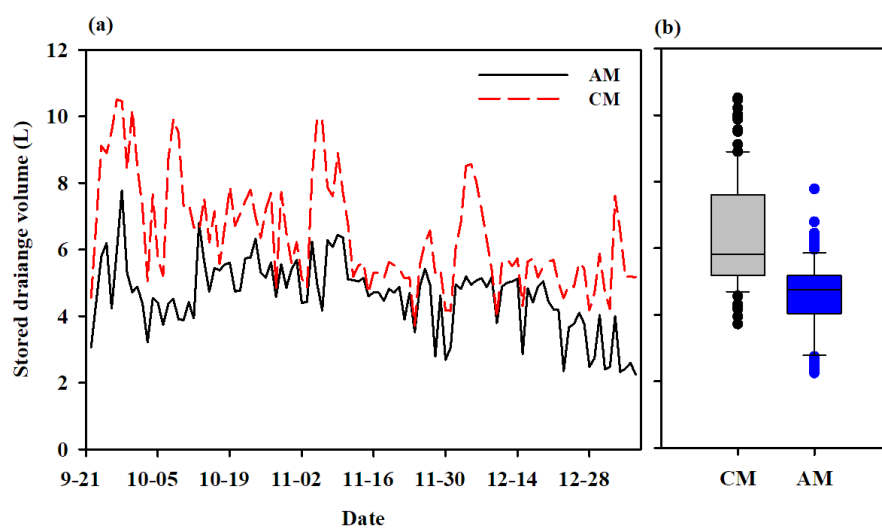


Fig. 3.6. Changes in stored drainage volume in the drainage tank (a) and box-plot comparison between conventional (CM) and alternative (AM) nutrient-replenishment methods (b) during the experimental period.

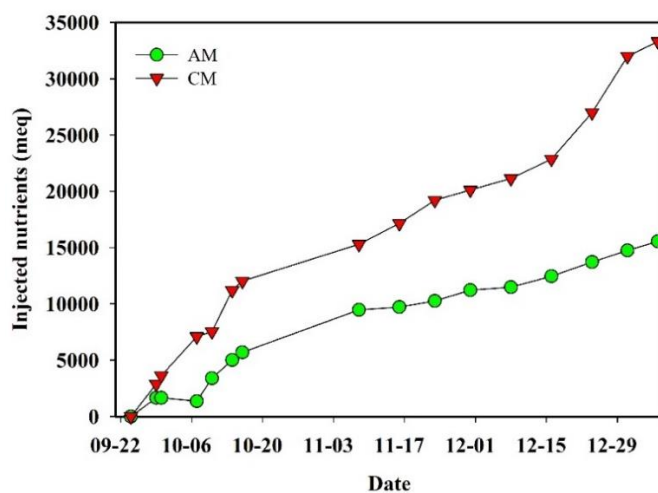


Fig. 3.7. Accumulated amounts of ions injected into the soilless culture systems with conventional (CM) and alternative (AM) nutrient-replenishment methods.

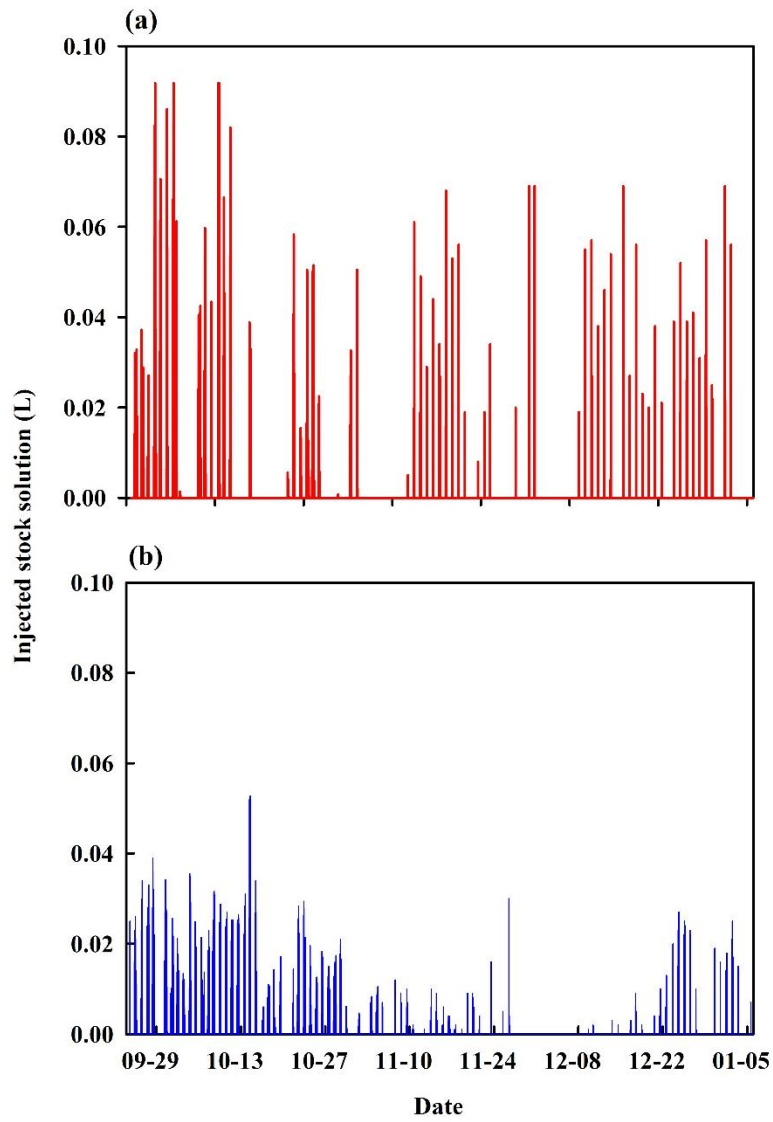


Fig. 3.8. Changes in volume of injected stock solution with conventional (CM, a) and alternative (AM, b) nutrient-replenishment methods.

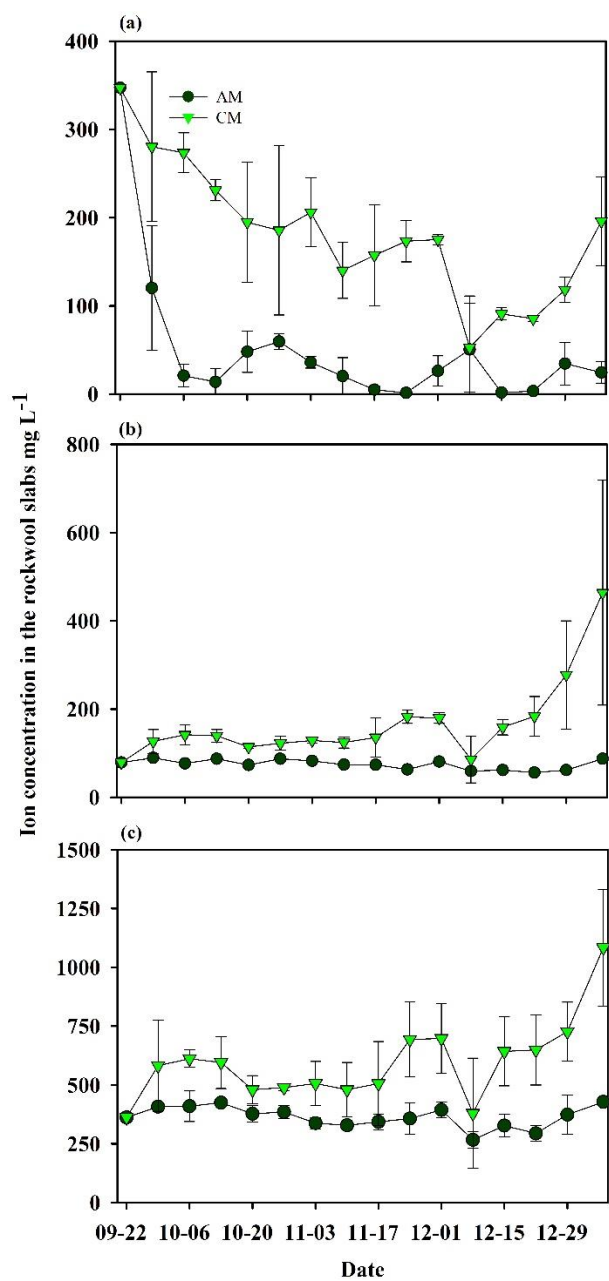


Fig. 3.9. Changes in nutrient concentrations of K⁺ (a), Mg²⁺ (b), and Ca²⁺ (c) in the root zone using the conventional (CM) and alternative (AM) nutrient-replenishment methods.

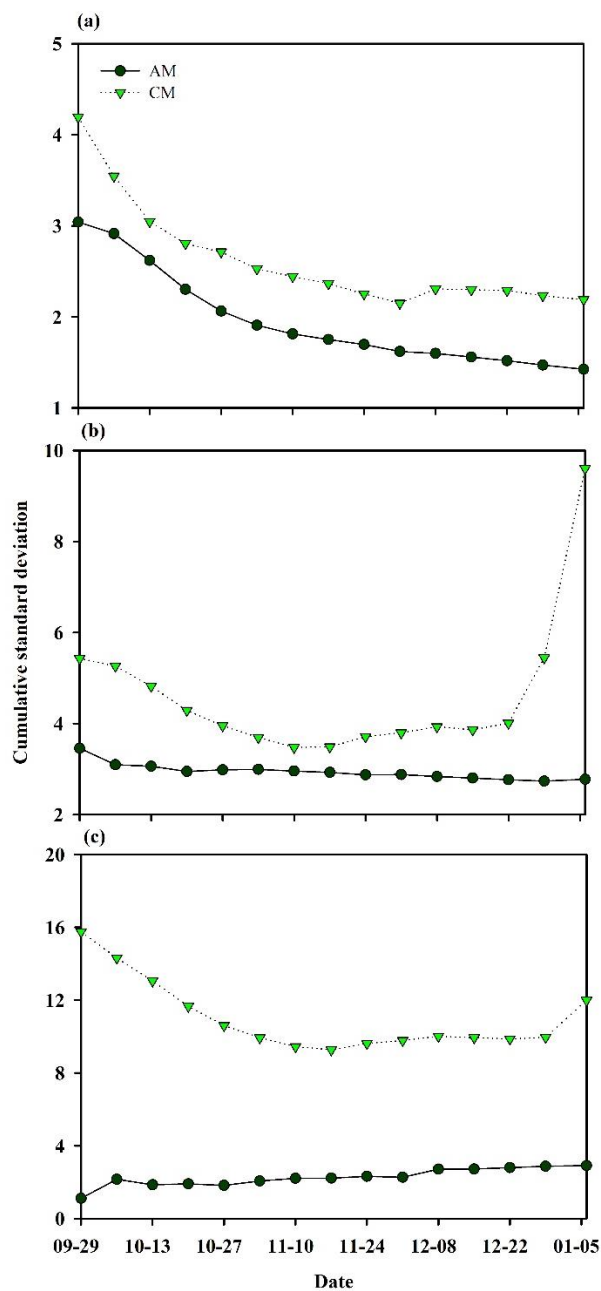


Fig. 3.10. Changes in cumulative standard deviation of nutrient concentrations of K^+ (a), Mg^{2+} (b), and Ca^{2+} (c) in the root zone using the conventional (CM) and alternative (AM) nutrient-replenishment methods.

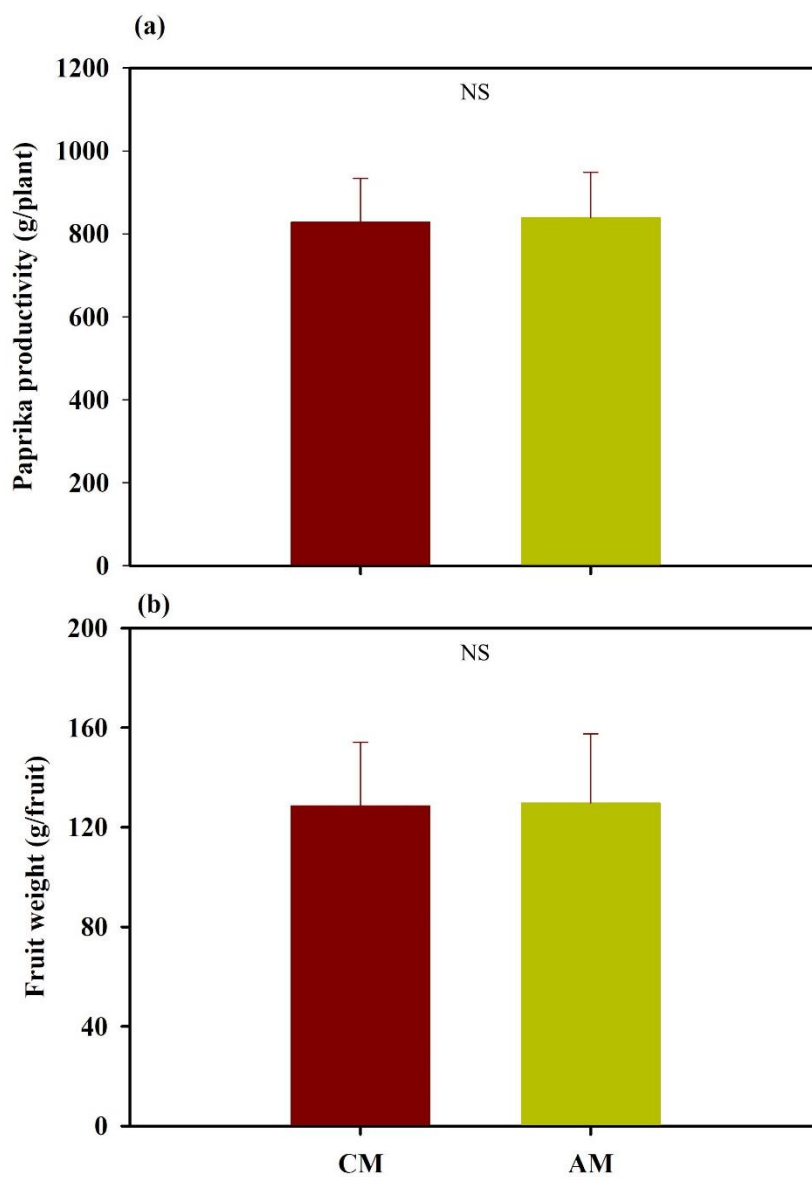


Fig. 3.11. Comparisons of total yield (a) and average fruit weight (b) of sweet pepper during the experimental period between conventional (CM) and alternative (AM) nutrient-replenishment methods (*t*-test). NS, not significant.

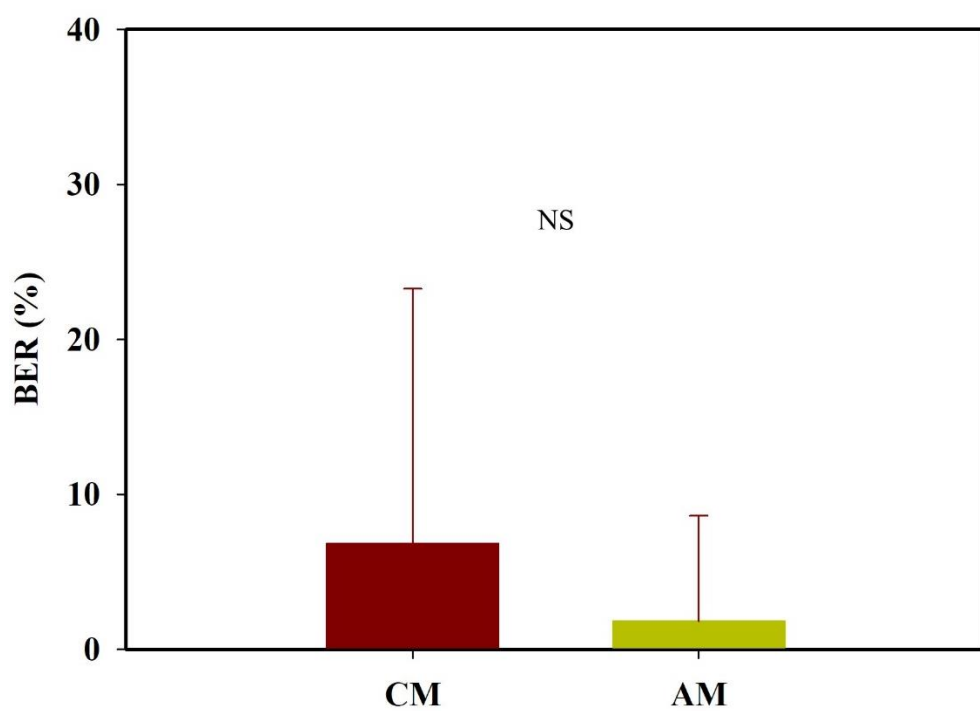


Fig. 3.12. Comparison of blossom-end rot of sweet pepper between conventional (CM) and alternative (AM) nutrient-replenishment methods (*t*-test). NS, not significant.

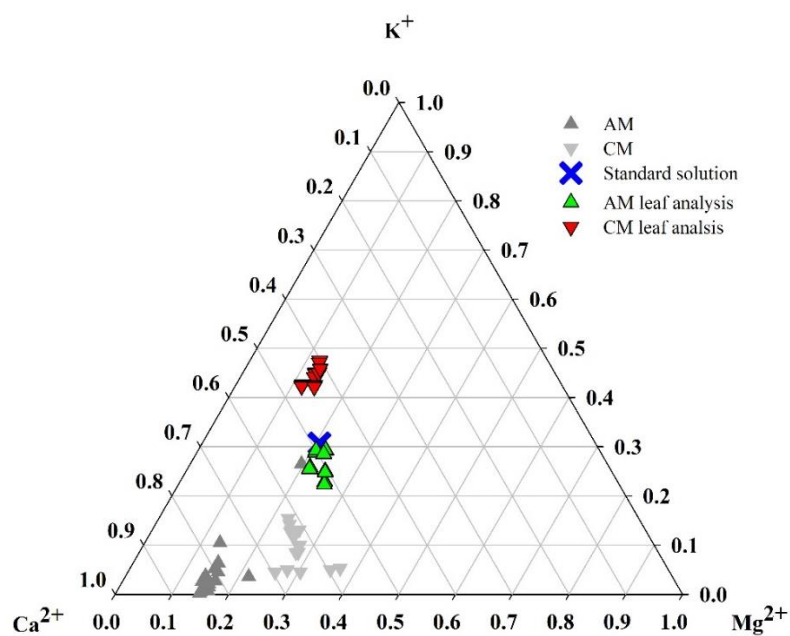


Fig. 3.13. Nutrient balance changes in the root zone and dried leaves using the conventional (CM) and alternative (AM) nutrient-replenishment methods.

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CHAPTER 4

Application of a Newly-developed Nutrient Replenishment Technique in Experimental- and Commercial-scale Electrical Conductivity-based Closed-loop Soilless Cultures

ABSTRACT

Technical solutions are needed to control nutrient concentration while minimizing fertilizer consumption in closed-loop soilless culture systems. A promising theoretically-derived alternative nutrient replenishment method that resulted in lower nutrient concentration variability and lower amount of fertilizer consumption than conventional methods was described in a previous study but no empirical validation of the method has been performed. This study applies the alternative nutrient replenishment technique to experimental and commercial-scale electrical conductivity (EC) -based closed-loop soilless cultures. Automated nutrient solution mixing modules and sweet pepper plants grown on rockwool slabs were used in the application of the method. Nutrient concentrations and plant productivity were measured in both the system using the alternative nutrient replenishment method and in the open-loop soilless culture system. During early experimental phases, rapid decreases in K^+ and $H_2PO_4^-$ were observed in the closed-loop system, but after the stock solution nutrient were adjusted, decreasing nutrient

levels stabilized and returned close to initial concentrations. The demonstration experiments showed no significant differences in quality or productivity of sweet pepper.

Additional keywords: nutrient control; nutrient management; closed-loop soilless culture; nutrient replenishment; steady-state control

INTRODUCTION

In general, sustainable management of resource use in a system can be ensured through the collection and reuse outflow products, which requires closed or semi-closed loop structures (Desmidt et al., 2015; Fiksel, 2003). However, in soil-based systems, it is difficult to create direct connections for the flow of nutrients and water (Granstedt, 2000), whereas in soilless culture systems, one can expect ideal control of nutrients and water, which maximizes productivity and creates physical blocks for resource outflow (Jones, 2005). These features indicate that soilless culture is a sustainable resource management model that can contribute to improving resource-use efficiency in agriculture. Despite this, soilless cultures have been regarded as a source of nutrient effluent at high concentrations due to common open-loop supply practices, which routinely produce drainage (Beerling et al., 2014; Noordwijk, 1990; Olympios, 1999; Raviv and Lieth, 2008).

While soilless culture has advantages with regards to precise control, root zone nutrients are more sensitive in soilless systems than they are in soil cultivation systems. Nutrient fluctuations caused by this structural limitation of the system itself have to be corrected frequently. The problem of nutrient output in water emission can be seen as a result of the conventional use of relatively simple nutrient management techniques that control fluctuations in the root zone by releasing a certain percentage of the drainage outside the system (Ku and Hershey, 1991; Noordwijk, 1990; Olympios, 1999).

A closed-loop soilless culture system that reuses discharged nutrients and water can minimize resource loss but the method is often accompanied by nutrient variations that result in a nutrient imbalance and instability in crop production (Hao and Papadopoulos,

2002; Kläring, 2001; Raviv and Lieth, 2008; Zekki et al., 1996). Because of the complications caused by nutrient variation in the root zone, an ideal management technique is to measure and calibrate nutrient components in real time (Gielsing et al., 2005; Gutierrez et al., 2007). Studies have focused on real-time measurements for a long time, but still this method has not replaced the conventional open-loop method in terms of technical stability or essential ion measurements (Bratov et al., 2010). This stagnation in methodology is not connected to the technological transition to the closed-loop system. One method that adjusts the concentration of total ions to a target level by measuring electric conductivity (EC) has been applied but problems related to maintaining a constant nutrient level have not been resolved, affecting the growth performance of plants (Ehret et al., 2005; Zekki et al., 1996).

Nutrient uptake in plants mostly follows the Michaelis-Menten equation (Clarkson, 1985; Cott et al., 2018; Le Bot et al., 1998). This means that plant systems can have steady-state solutions depending on the input variables and parameters (Golicnik, 2011) and that the nutrient concentration in the plant root zone can converge to a specific value after a certain period of time. An appropriate input to a system is required to reach a steady state. In an EC-based closed-loop soilless culture system, nutrients are introduced to the system through the replenishment of standard stock solution. But under actual cultivation conditions, EC fluctuations are observed (Hao and Papadopoulos, 2002; Massa et al., 2011; Signore et al., 2016). This means that the conventional nutrient replenishment method is unfavorable for the steady state control of nutrients.

Chapter 3 has analyzed the nutrient variation in closed-loop systems, leading to a new problem definition for nutrient variation in the system and aiding in the formulation of an alternative nutrient replenishment technique that confirmed that maintaining the total nutrient concentration around an initial concentration is associated with relatively stable changes in individual nutrient concentration. In addition to stabilizing an EC-based closed-loop soilless culture system, it is also important to control individual nutrient concentrations within a target range or at a target value.

This study aims to apply the alternative nutrient replenishment technique to nutrient control in experimental- and commercial-scale EC-based closed-loop soilless cultures and to verify its effect on plant production.

MATERIALS AND METHODS

Nutrient control technique

The three steps in the nutrient balance control process applied in this experiment is shown in Fig. 1: first, nutrient model calibration was performed; then, a short-term increase process was performed for the nutrients exhibiting large decreases in concentration; finally, the steady state solution of each nutrient was calculated based on the calibrated model and used to adjust the stock solution composition. A model generated in Chapter 2 was used for model calibration alongside progress curve analysis. The injection amount needed for a short-term increment for a largely decreased nutrient was calculated based on the Yamazaki n/w method (Anpo et al., 2018). The alternative nutrient replenishment technique proposed in Chapter 3 was used in the closed-loop soilless culture system.

Cultivation conditions for experimental-scale systems

A Venlo-type greenhouse at the experimental farm of Seoul National University, Suwon, Korea (37.3° N 127.0° E) was used for the experimental scale systems. Three sweet pepper (*Capsicum annuum* L. ‘Derby’) plants were grown in a rockwool slab and seven slabs were used per row. Four cultivation lines were installed in the greenhouse, each consisting of an independent closed-loop soilless culture system with a mixing tank, drainage tank, and stock solutions. The initial composition of the stock solution was prepared based on the PBG nutrient solution of the Netherlands. In the greenhouse, daytime temperatures of 25 – 35°C and nighttime temperatures of 15 – 22°C were

maintained. Experiments were carried out between April 2016 and July 2016. Open- and closed-loop soilless culture system were applied for nutrient control. Target EC for the open-loop soilless culture system was set to $2.6 \text{ dS}\cdot\text{m}^{-1}$. An integrated solar radiation method was applied for irrigation control. Composition of the prepared standard nutrient solution composition was $14.17 \text{ meq}\cdot\text{L}^{-1}$ of NO_3^- , $1.14 \text{ meq}\cdot\text{L}^{-1}$ of H_2PO_4^- , $5.92 \text{ meq}\cdot\text{L}^{-1}$ of K^+ , $8.85 \text{ meq}\cdot\text{L}^{-1}$ of Ca^{2+} , $3.17 \text{ meq}\cdot\text{L}^{-1}$ of Mg^{2+} , and $3.20 \text{ meq}\cdot\text{L}^{-1}$ of SO_4^{2-} as macro elements; and $0.038 \text{ meq}\cdot\text{L}^{-1}$ of Fe^{2+} , $0.020 \text{ meq}\cdot\text{L}^{-1}$ of Zn^{2+} , $0.003 \text{ meq}\cdot\text{L}^{-1}$ of Cu^{2+} , $0.021 \text{ meq}\cdot\text{L}^{-1}$ of Mn^{2+} , and $0.001 \text{ meq}\cdot\text{L}^{-1}$ of MoO_4^{2-} as micro elements. The same nutrient solution mixing module from Chapter 3 was used in this experiment. The effect of tap water was minimized by using four reverse osmosis filters (150GPD, Waterpia, Korea). Detectable nutrient concentrations in tap water were $0.001 \text{ meq}\cdot\text{L}^{-1}$ of Na^+ at the time of initial installation. A solar radiation-based irrigation control was applied. When the cumulative radiation amount reached $100 \text{ J}\cdot\text{cm}^{-2}$, 150 mL of the nutrient solution was supplied to each plant. A drainage ratio of approximately 30% was maintained by setting the irrigation amounts and cumulative radiation values depending on meteorological conditions.

Cultivation conditions for commercial-scale systems

A Venlo-type greenhouse at the commercial farm of ‘Hwasung 21’ (Hwasung, Korea, lat. 37.0°N , long. 126.8°E) was used for the commercial-scale systems. Sweet pepper plants (*Capsicum annuum* L. ‘Veyron’) were arranged in two rows for each lane, and 126 plants were planted in each row and allocated for closed-loop and open-loop system

treatments, respectively. The experiments were carried out between May 2016 and July 2016. The nutrient solution mixing module which was installed in the commercial farm has the same components as the small-scale experiment with the addition of a UV sterilizer in the mixing tank circulation pipe. Thus, when the process of nutrient solution mixing occurs in the mixing tank, the reused nutrient solution is sterilized. To assess the effect of sterilization in this experiment, biological contamination level was assessed by using a portable ATP water test kit (3M Clean-Trace) at the end of each experiment. In order to confirm the control effect of nutrient input by stock solution, the effect from tap water was minimized by using eight reverse osmosis filters (150GPD, Waterpia, Korea). The same prepared standard nutrient solution and irrigation management methods were applied to the commercial scale system that were used in the experimental scale systems.

Measurement of fruit yield and analysis of nutrient content in leaves and fruits

Total fruit yield, fruit weight, and the ratio of blossom-end rot (BER) to total number of fruit yield per sweet pepper plant were measured. At the end of the experiment in the commercial scale farm, 3 leaves (including petiole) at the fourth and fifth nodes from the top of the stem and at the second and third nodes from the bottom of the stem were collected respectively from each treatment. To analyze fruit nutrient content, matured fruits (three fruits per treatment) were collected in the middle and last harvest of the experiment. The fruits were dried at 70°C in an oven until they reached a constant weight. Leaves and fruits were washed in tap water and leaves were dried for 48 h at 70°C in the oven. The dried leaves and fruits sampled were ground and 0.5 g of each sample was

digested with concentrated nitric acid. Then, 1 ml of concentrated perchloric acid was added to maintain a set solution temperature of 180°C and to accelerate the digestion process on a 90°C hot plate for about one hour until a clear solution was obtained. After the digestion, the tube was cooled and topped up with 25 ml deionized water, and the total contents of K, Ca, Mg, P, and S from leaves were determined by the inductively coupled plasma optical emission spectrometer (ICP-730ES, Varian, Mulgrave, Australia). Total-N was measured by Kjeldahl (Kjeltec 8400, Foss, Sweden).

Nutrients analyses and statistics

To observe changes in nutrient concentrations and ratios in the root zone of the soilless culture system, samples of nutrient solution in the rockwool slabs were extracted using a syringe. The collection points of the nutrient solution in the rockwool slab were randomly selected in order to obtain representative samples of the overall concentration in the root zone. Five 10-mL samples of root zone nutrient solution were collected for each extraction, yielding a total of 50 mL of sample. Three samples per treatment were collected each week. K^+ , Ca^{2+} , Mg^{2+} , S (SO_4^{2-}), P ($H_2PO_4^-$) were analyzed using an inductively coupled plasma optical emission spectrometer (ICP-730ES, Varian, Mulgrave, Australia). The concentration of NO_3^- was measured with a spectrophotometer (PhotoLab, 6100 [VIS], WTW, Weilheim, Germany). SAS software (version 9.2, SAS Institute, USA) was used for statistical analysis.

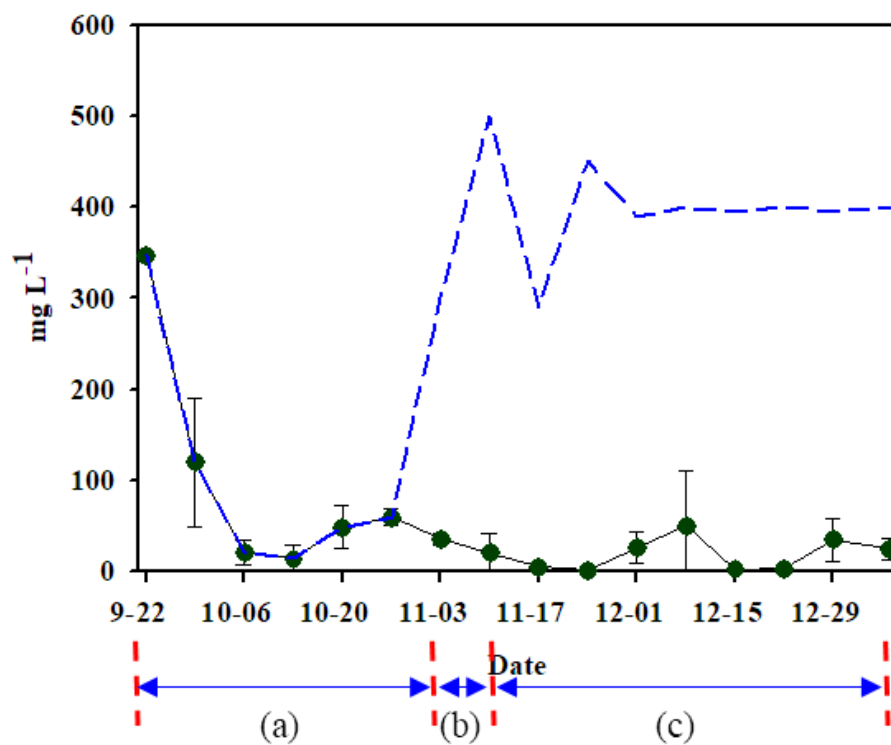


Fig. 4.1 A conceptual diagram of the nutrient-balance control technique. (a) Online-parameter estimation for nutrient uptake model (progress curve analysis); (b) short-term increment of largely decreased nutrient; and (c) application of adjusted stock solution composition. dashed line indicates desired nutrient changes.

RESULTS AND DISCUSSION

Experimental-scale cultivation experiments

In the closed-loop soilless culture system, an initial rapid decrease was observed in the K^+ cation before nutrient adjustment (Fig. 4.2) that was not observed at later time points. The stabilization of K^+ can be interpreted as an indication that the addition of the stock solution according to progress curve analysis helps stabilize the system.

In the case of anions, $H_2PO_4^-$ tended to decrease rapidly following an initial increase in both the open-loop and closed-loop soilless culture systems (Fig. 4.3). However, in the case of the open-loop soilless culture system, no decrease was observed below the initial value, and in the case of the closed-loop system, the decrease continued until the adjustment point. The same correction procedure was used for the stock solution and short-term recovery for $H_2PO_4^-$ as was used for K^+ . In the case of SO_4^{2-} , an increase was observed that gently changed to continuous decrease after nutrient adjustment. This suggests that control for the decrease of nutrients in the closed-loop soilless culture system must depend on the absorption rate of the plants, unlike the nutrient replenishment control.

An increase in divalent ions such as Ca^{2+} , Mg^{2+} , and SO_4^{2-} in a closed-loop soilless culture is commonly observed (Sonneveld and Voogt, 2009; Zekki et al., 1996). This can be seen as the result of the selective nutrient uptake by the plants (Steiner, 1980). Standard nutrient solutions have been developed in consideration of each nutrient requirement ratio, chemical stability, and yield of plants through plant nutritional studies (Bautista et al., 2009; Hoagland and Arnon, 1950; Jones, 1982; Steiner, 1980). Even though previous standard

nutrient solutions were developed with information about nutrient requirement ratios, the input nutrient ratios for a steady state could be different than predictions because of nonlinear rate change in nutrient uptake. While previous studies have observed nutrient changes which could be interpreted as a steady state (Neocleous and Savvas, 2018; Savvas, 2002; Savvas et al., 2007), they have not discussed results in terms of a steady state control technique.

Nutrient ratios are shown in Fig. 4.4. In the case of nutrient concentrations, relatively large fluctuations were observed, but the level of the fluctuation tended to decrease when data were converted to a nutrient ratio. In the case of the closed-loop soilless culture system, the nutrient input to the system is relatively low, which means that changes recorded before the calibration in the first step were high when compared with the open-loop soilless culture system. After nutrient adjustment, the ratios returned closed to initial values.

According to Ehret et al. (2005) and Zekki et al. (1996), it takes a certain period of time until changes in nutrient concentrations in the closed-loop soilless culture system affect crop growth. So, it is unlikely that the exposure of plants to fluctuations in nutrient concentration and ratios before the nutrient adjustment would affect the plants. No statistically significant differences were found in the productivity, sugar content, blossom end rot, or incidence of sweet peppers across the two experiments (Fig. 4.5).

Commercial-scale cultivation experiments

Result from the commercial scale experiments are shown in Fig. 4.6 and Fig. 4.7. When evaluating cation patterns in the closed-loop soilless culture system, a similar result was

seen to that reported for the experimental-scale experiment: a rapid decrease was observed in K^+ before the adjustment point. In contrast, however, K^+ did not decrease after the nutrient adjustment point and instead stayed relatively stable.

For anion analyses, $H_2PO_4^-$ decreased rapidly (Fig. 4.7) and the same procedure described above was carried out for the correction of the stock solution. After a short-term increase in concentration, the nutrient then stabilized in a similar manner to K^+ . SO_4^{2-} concentration until it was adjusted and then a slight decrease was observed.

Nutrient concentration ratios are reported in Fig. 4.8. The ratios of cations and anions in open-loop soilless culture were lower than those in closed-loop soilless culture. However, in the case of the closed-loop soilless culture system, the change in the direction of change for K^+ (decrease) and Ca^{2+} (increase) occurred in the cation until the initial analysis, but returned to the initial ratio after adjustment. A similar pattern was observed for anions: changes of SO_4^{2-} and $H_2PO_4^-$ were reversed and ratios were returned to their starting points after adjustment.

Changes in nutrient concentrations and ratios observed in the closed-loop soilless culture system did not have statistically significant effects on sweet pepper fruit productivity, sugar content, or navel rot incidence (Fig. 4.9). Leaf analysis showed that there was a significant difference in total nitrogen (T-N) and lower leaf Mg content in the upper leaves, but values were not high enough to constitute a deficiency or excess according to the sweet pepper growing manual (Ministry of Agriculture, 2005). The difference between T-N and Mg was 0.15% p and 0.11% p, respectively (Table 4.1). In the case of sweet pepper harvested at the time of the first nutrient correction, there was a

significant difference between Mg and S, which was about 0.02% p. There was no significant difference in ion content corresponding to late harvest after the nutrient correction. Indirect measurement of microbial concentration through an ATP activity test on the nutrient solution in the root zone showed a significantly lower value in closed-loop soilless culture system than open-loop systems (Fig. 4.10). In this study, the UV sterilizer was installed in path of the circulation pipe in the mixing tank. Therefore, the sterilization of the reused nutrient solution was made whenever mixing occurred for the irrigation water. While closed-loop soilless culture systems can cause contamination of the reused nutrient solution itself because it reuses drainage from plant rhizosphere and greenhouse environments, the UV sterilization systems can act as inactivation kinetic constants for microorganisms within a system depending on their type and condition (Labas et al., 2005). In this study, the UV sterilizer was installed on the circulation pipe of the mixing tank, but it could have acted as an inactivation kinetic constant in the closed-loop soilless culture circulation system.

Overall, large variations in several nutrient concentrations were observed in both experimental- and commercial-scale open-loop soilless culture experiments than in closed-loop soilless culture experiments, but when looking just at nutrient ratios, the variability in open-loop systems was lower than in closed-loop soilless systems. Variations in nutrient concentration in open-loop soilless systems have been reported to be a result of differences between nutrient uptake concentration and supply nutrient concentration (Noordwijk, 1990). Variations in nutrient concentration in open-loop systems observed in this study could be interpreted in the same way. Since the nutrient supply was based on an EC

control—meaning that the variations affected total nutrient concentration—a similar trend in nutrient concentrations could occur, leading to lower variability in nutrient ratios. However, the purpose of the alternative nutrient replenishment technique in the closed-loop soilless culture system was controlling the average steady state in total nutrient concentration. Therefore, the overall aspect of nutrient concentration changes was distinguished from the changes in the open-loop soilless culture system.

In conclusion, the alternative nutrient replenishment method tested here can be used to achieve a controlled steady-state while minimizing nutrient consumption. In open-loop soilless culture systems, on the other hand, nutrients are controlled by adjusting the amount of irrigation.

Table 4.1. Comparison of nutrient contents in leaves and fruits of sweet pepper in open-loop and closed-loop cultures.

Position	Treatment	T-N	P	K	Ca	Mg	S
Top	Open	3.52±0.06	0.36±0.06	6.14±0.81	3.15±0.12	0.44±0.04	0.52±0.03
	Closed	3.67±0.02	0.42±0.03	5.38±0.33	3.54±0.22	0.51±0.05	0.57±0.02
	<i>t</i> - test	*	NS	NS	NS	NS	NS
Bottom	Open	2.59±0.15	0.21±0.03	5.72±0.83	4.53±0.17	0.58±0.01	0.42±0.07
	Closed	2.65±0.26	0.25±0.05	6.61±0.65	4.46±0.27	0.69±0.04	0.52±0.05
	<i>t</i> - test	NS	NS	NS	NS	*	NS
Fruit (6.14)	Open	2.01±0.14	0.38±0.02	2.66±0.12	0.09±0.01	0.11±0.009	0.23±0.01
	Closed	1.85±0.09	0.33±0.02	2.36±0.34	0.08±0.01	0.09±0.003	0.21±0.01
	<i>t</i> - test	NS	NS	NS	NS	*	*
Fruit (7.12)	Open	2.16±0.18	0.38±0.03	2.62±0.13	0.07±0.01	0.11±0.011	0.22±0.03
	Closed	1.99±0.08	0.37±0.01	2.63±0.41	0.08±0.02	0.11±0.013	0.23±0.01
	<i>t</i> - test	NS	NS	NS	NS	NS	NS

$p < 0.05$ by *t*-test.

NS, not significant; *, significant at $p = 0.05$.

The numbers in parentheses indicate the date of harvest.

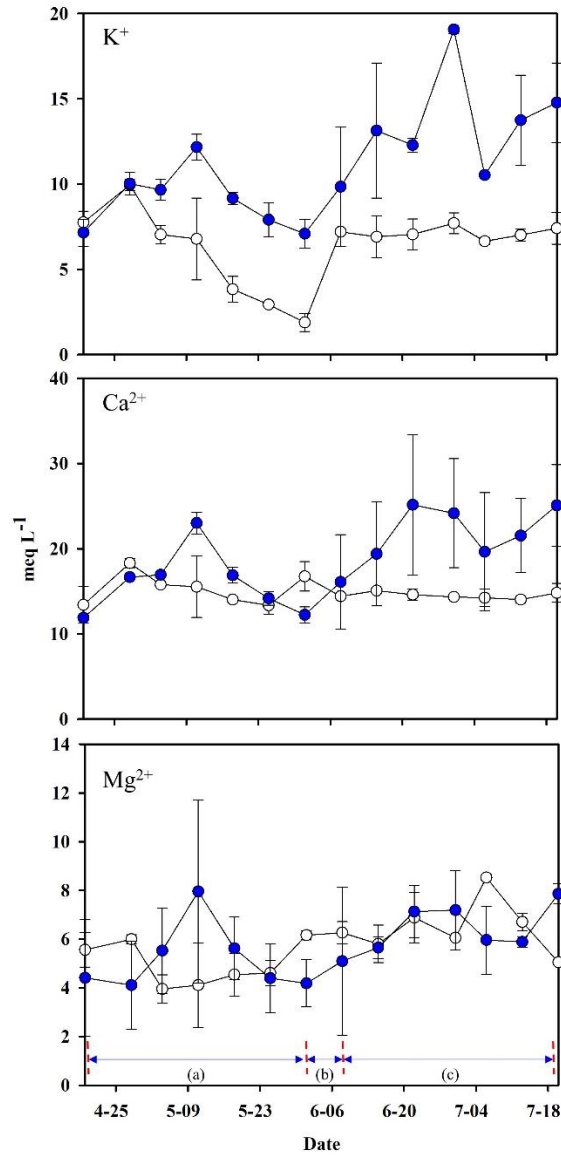


Fig. 4.2. Cation concentration changes with the nutrient control in an experimental scale experiment. (a) Online-parameter estimation for nutrient uptake model (progress curve analysis); (b) short-term increment of largely decreased nutrient; and (c) application of adjusted stock solution composition. ● and ○ indicate open- and closed-loop soilless cultures, respectively.

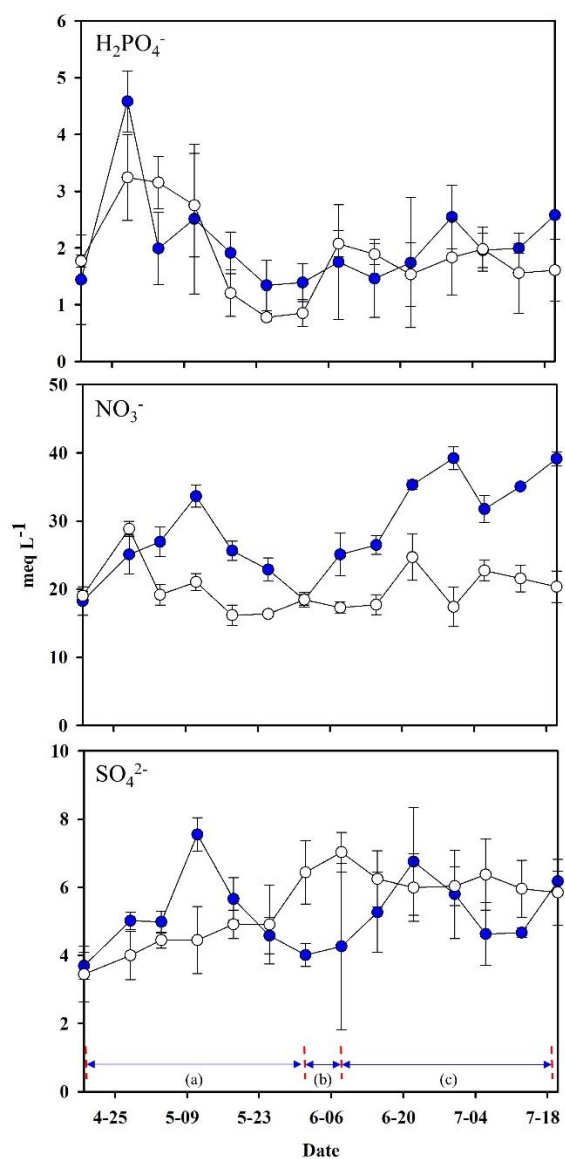


Fig. 4.3. Anion concentration changes under nutrient balance control in a small scale experiment. Online-parameter estimation for nutrient uptake model (progress curve analysis) (a); short-term increment of largely decreased nutrient (b); and application of adjusted stock solution composition (c). ● and ○ indicate open- and closed-loop soilless cultures.

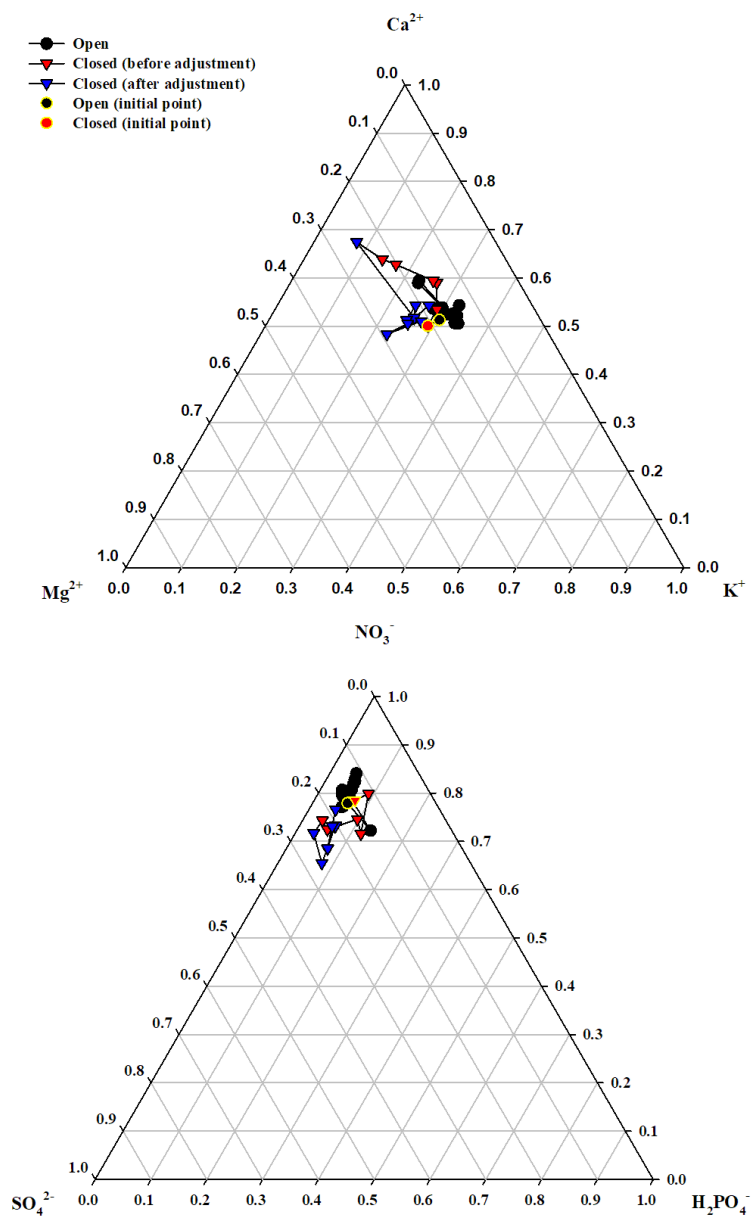


Fig. 4.4. Nutrient balance changes under nutrient balance control in an experimental-scale experiment.

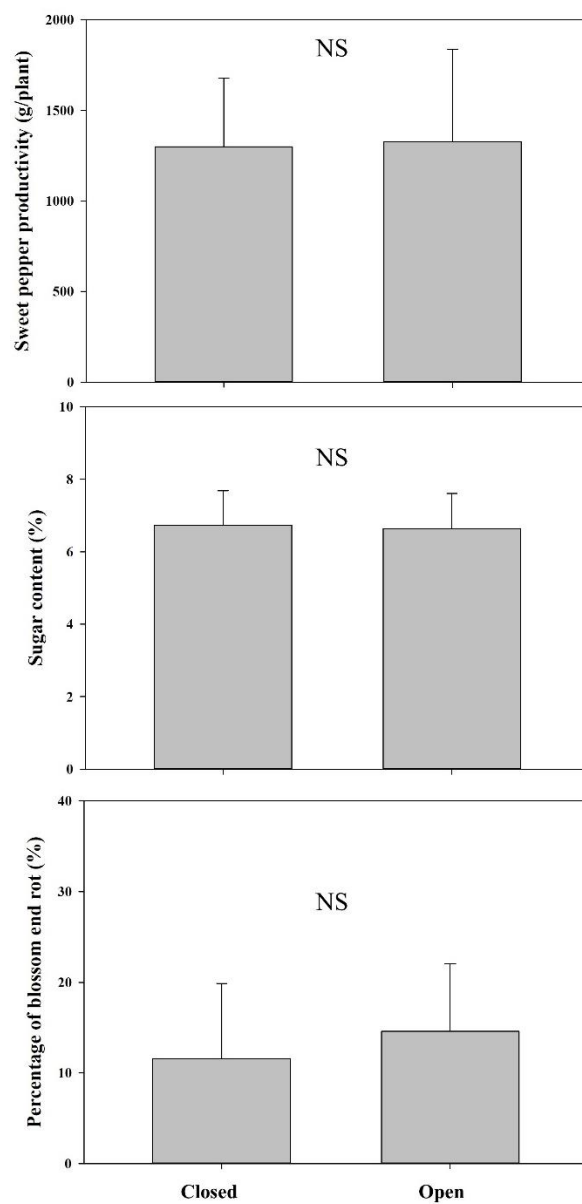


Fig. 4.5. Comparisons of the productivity, sugar content, and percentage of blossom end rot of sweet pepper between the closed-loop soilless culture with the nutrient balance control and the open-loop soilless culture in an experimental-scale experiment (*t*-test). NS: not significant.

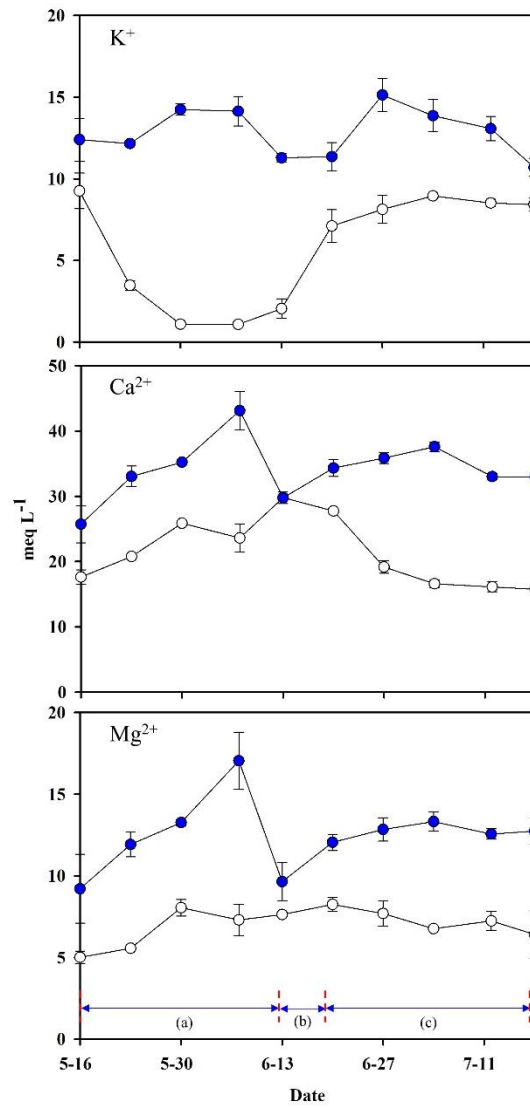


Fig. 4.6. Cation concentration changes with the nutrient balance control in a commercial-scale greenhouse. Online-parameter estimation for nutrient uptake model (progress curve analysis) (a); short-term increment of decreasing nutrient (b); and application of adjusted stock solution composition (c). ● and ○ indicate open- and closed-loop soilless cultures.

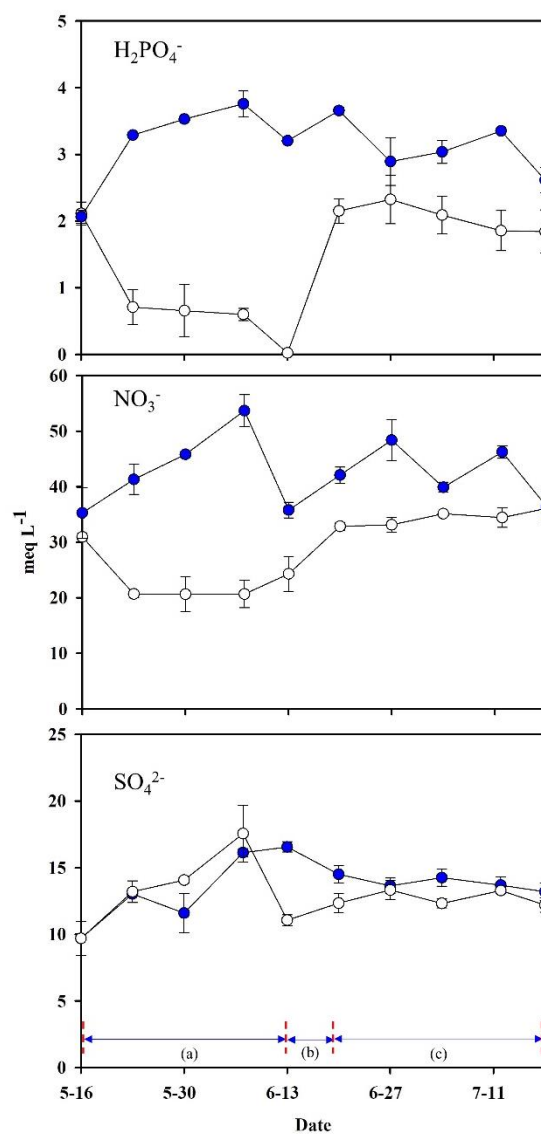


Fig. 4.7. Anion concentration changes under nutrient balance control in commercial-scale experiment. Online-parameter estimation for nutrient uptake model (progress curve analysis) (a); short-term increment of largely decreased nutrient (b); and application of adjusted stock solution composition (c). ● and ○ indicate open- and closed-loop soilless cultures.

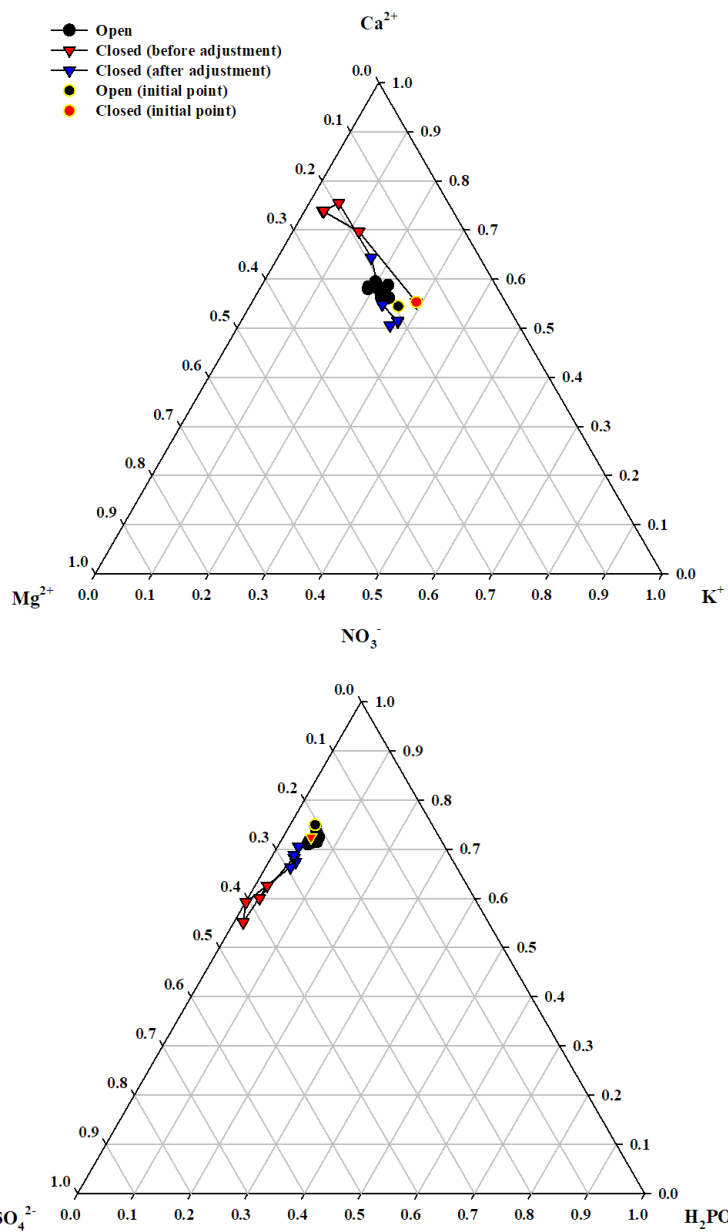


Fig. 4.8. Nutrient balance changes under nutrient balance control in the commercial scale experiment.

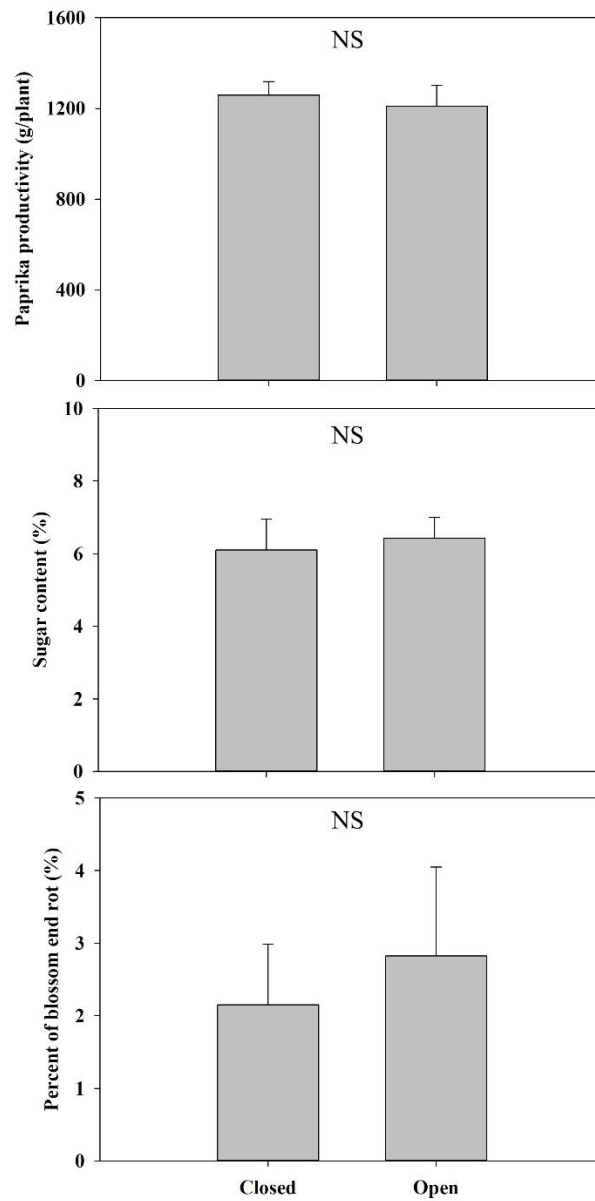


Fig. 4.9. Comparisons of the productivity, sugar content, and percentage of blossom end rot of sweet pepper between the closed-loop soilless culture with the nutrient balance control and the open-loop soilless culture in a commercial-scale greenhouse (*t*-test). NS: not significant.

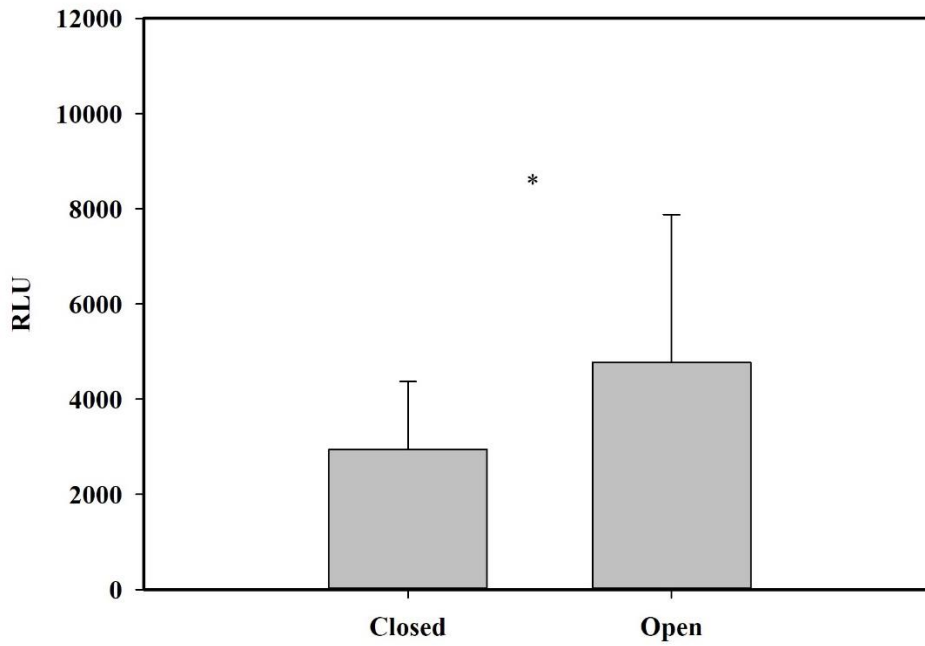


Fig. 4.10 Comparison of relative light unit (RLU, microbial concentrations) values in the closed- and open-loop soilless cultures at the end of the experiment. An asterisk indicates significant differences ($p < 0.05$ by t - test).

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CONCLUSIONS

EC-based soilless culture systems are unable to detect the changes in individual nutrient concentrations affected by environments and a systematic technique for nutrient control in soilless culture has not been presented yet. In addition, the closed-loop soilless culture system is potentially accompanied by biological contamination due to the reuse of nutrient solution. The main goal of this study was to contribute to the technical systematization of closed-loop soilless cultures for spreading the system.

The number of colony forming unit (CFU) was investigated to distinguish the biological contamination of the recycled nutrient solution from the whole system contamination. CFU in four commercial greenhouses applied with open- and closed-loop soilless culture were investigated. Greenhouses using closed-loop soilless culture were investigated to be more advantageous condition for restraining microbial proliferation inside the greenhouse due to active drainage collection.

Through this study, theoretical models of EC-based soilless culture system were constructed and this provided a system-wide perspective for integrative interpretations on the nutrient variations in soilless culture system. The theoretical analyses of this study have defined the problem of EC variation in the system, theoretically predicted a solution for technical improvement in nutrient concentration variation, and derived a nutrient control technique in EC-based closed-loop soilless culture system. The EC variations in closed-loop system defined as a problem of the nutrients replenishment method and this was eventually turned into a problem of proportional control.

Based on the problem definition, an alternative nutrient replenishment method (AM) could be presented. This minimized the error between total nutrients input and total nutrients uptake and consequently reduced fertilizer input compared to conventional nutrient replenishment method (CM) in the cultivation experiment. Furthermore, this result does not only mean that it was concluded as a problem of proportional control, but it means that more advanced techniques in control engineering could be applied through accurate problem definition in the closed-loop soilless culture system.

Finally, the nutrient control technique under the AM applied EC-based closed-loop soilless culture system which was derived from the theoretical analyses was demonstrated under the experimental and commercial scale greenhouse conditions and compared with the open-loop system. The demonstration experiment showed no significant differences in quality and productivity of sweet pepper and the convergence of nutrient changes within target values were observed.

These results mean, unlike the previous approaches in nutrient management, that the theoretical explanation on the nutrient variation and control technique and its technical expansion can be made. As a result, the theoretical model-based approach presented in this study is expected to contribute to the technical systematization of the closed-loop soilless culture cultivation system.

ABSTRACT IN KOREAN

일반적으로 수경재배 시스템의 양분 관리 기술은 이온의 총 당량 농도에 비례하는 전기전도도(EC, electrical conductivity)를 기반으로 구축되어 왔다. 그러나 이러한 방식은 환경에 따라 동적으로 변하는 개별 양분의 농도 변동을 감지 할 수 없으며, 양분 변동을 제어하기 위한 체계적인 기술은 아직 제시되지 못하고 있다. 또한 부수적으로 배액의 재사용으로 인한 생물학적 오염으로 인한 잠재적인 문제를 동반할 수 있다. 순환식 수경재배 시스템이 비순환식 수경재배 시스템을 대체하기 위해서는 기술적 체계화를 통한 운용 안정성의 확보가 필요하다. 본 연구는 순환식 수경재배의 기술 체계화를 목적으로 EC 기반 순환식 수경재배 시스템의 양분 변동 해석을 위한 이론적 모델을 구축하였으며, 이론적 분석을 통해 양분 제어 기술을 도출하고 실험적으로 실증하였다. 기초적으로는 순환식 수경재배 방식 적용에 따른 생물학적 오염 문제를 재사용 양액의 생물학적 오염과 온실 내부로의 양액 배출로 인한 전체 재배 시스템의 오염을 체계적으로 구별하기 위해 온실에서의 집락 형성 단위(CFU)를 조사하였다. 또한 시스템 내 양분의 변화 양상을 파악하고 이를 기반으로 이론적 해석으로 확장하기 위해 순환식 수경재배 시스템에서의 양분 농도와 비율 변화를 조사하였다. 먼저, 순환식과 온실 내부로 배액을 배출하는 비순환식을 비교한 결과, 적극적인 배액을 수집하는 순환식 시스템이 온실 내부의 미생물 증식 억제에 있어서는 유리한

조건으로 작용할 수 있음을 확인하였다. 양분 변화 조사를 통해서도 양분 농도의 동적인 변동이 관찰되는 반면 양분의 균형 변화는 일정한 경향이 관찰됨을 확인하였다. 이를 이론적 분석으로 확장하기 위하여, EC 기반 수경재배 시스템의 이론적 모델을 구축하였으며 양분 균형 변화에 대한 기초 조사를 이론 및 실험적 분석으로 확장하였다. 분석 결과, 양분 흡수 농도에 대해 무작위 외란이 적용된 조건에서도 장기적으로 양분 간의 균형이 제어 목표에 수렴할 수 있음을 확인하였다. 또한, 순환식 수경재배에서의 EC 변동의 문제를 해결하기 위하여 대안적 양분 보충 방법을 제시하였다. 대안적 양분 보충 방법은 기본적으로 시스템 내 전체 양분의 흡수량 변화 추종 수행을 목표 기능으로 도출하였다. 순환식 수경재배 시스템의 시뮬레이션 분석을 통해 관행 양분 보충방법과 대안적 양분 보충방법 적용에 따른 효과를 이론적으로 비교하였다. 또한, 이러한 효과의 실제 재배 시스템에서의 재현 여부를 확인하기 위해 실증 실험을 수행하였다. 시뮬레이션에서의 예측과 같이 전체 양분 투입량과 전체 양분 흡수량과의 오차가 최소화되며, 관행의 양분 보충 방법에 비해 비료의 사용량을 절감을 확인할 수 있었다. 관행 방식에 비해 균일한 양분 투입이 관찰될 수 있음을 확인하였다. 또한 평균적 정상상태로 추정되는 양상의 양분 농도 변화가 관찰되었다. 마지막으로, 대안적 양분 보충 방법이 적용된 EC 기반 순환식 수경재배 시스템 하에서 이론적 분석으로부터 도출된 양분 제어 기술을 실증하였다. 각각 실험 및 상업적 규모의 파프리카 온실 실증 실험에서 순환식과

비순환식에서의 파프리카 품질과 생산성에서 유의적인 차이를 관찰할 수 없었으며, 순환식에서 양분 변화가 목표값으로 수렴하는 것을 확인하였다. 본 연구에서 구축된 이론적 모델을 통해 EC 기반 수경재배 시스템의 양분 변동을 분석하였다. 양분 제어 기술은 이론적 분석을 바탕으로 도출되었으며 재배 실험을 통해 실증하였다. 이러한 접근 방식은 추후 기술 심화 및 확장 가능하며, 결과적으로 순환식 수경재배 시스템의 기술적 체계화에 기여할 수 있을 것으로 판단된다.

추가주요어: 양분 흡수 모델; 양분 이용 효율; 식물 무기 영양; 순환식 수경재배 시스템; 양분 제어

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APPENDIX
SUPPORTING INFORMATION ON THEORETICAL ANALYSIS

Supporting Information Section 1. Effects of the conventional nutrient-replenishment method (CM) on stored volume of drainage (Chapter 3)

A closed-loop soilless culture system is structurally closed to resource flow. In the case of water, the amount of input is limited by the physical size of the system. However, nutrients can be introduced without restriction. Closed-loop soilless culture systems control total nutrient concentration through electrical conductivity (EC) measurements. That is, the excess nutrient input into the system due to accumulation of errors between nutrient uptake rate and rate of nutrient supply produces a possibility that the system will become unstable during the later EC adjustment of the system.

Fig. A.2b shows the change in concentration in the root zone at each time step and the amount of nutrients required to return concentrations to initial values when the simulation is performed under the conventional nutrient-replenishment method. The amount of nutrient solution to be fed into the system to adjust current nutrient concentrations to the initial concentration of the system requires about 100 times more irrigation volume per event after 60 days in the simulation. This results in overflow of the drainage tank and influences resource use efficiency.

In the case of change of drainage volume, as in Fig. A.2a, drainage volume often exceeds the irrigation volume per event. If, as described in the nutrient solution mixing control section, the EC of the drainage could not be adjusted to the target concentration by dilution, the stock nutrient solution could not be supplied. In the case of an EC-based closed-loop soilless culture system, the correction effect of nutrient variations in the system can be achieved by injecting stock solution into the system. This feature can serve

as a limiting factor in the system's ability to correct for nutrients in an actual closed-loop soilless culture system.

Supporting Information Section 2. Effects of the conventional nutrient-replenishment method (CM) on stock solution injection frequency and amount of stock solution consumption (Chapter 3)

In a soilless culture system in which total nutrient replenishment is carried out by applying the CM, the total amount of nutrients in the system increased, as the nutrient input amount is frequently larger than the plant absorption amount.

The volume of water circulating through the system is physically constrained by the size of the system. However, as the total amount of nutrients in the system continuously increases, the total concentration of the system increases. As a result, the concentration of the drainage increases, and eventually irregular injection events of stock solution occurred.

This effect is also observed in Fig. A.3. In the box plot in Fig. A.3b, the distribution of injected stock solution volume in the CM is very non-uniform compared to that of AM1. On the other hand, in AM1, the amount of stock solution is relatively uniform throughout the simulation period compared with the CM. EC-based soilless culture systems achieve nutrient correction through replenishment of the stock solution. However, in the distribution of stock solution input of the CM, such replenishment can be disadvantageous in terms of correction of nutrients, which can be regarded as a technical uneasiness factor. The AM, as confirmed through simulation, can follow the amount of nutrients absorbed in the soilless culture system. That is, the excess amount of nutrients is less than in the CM system. Thus, even in a completely closed-loop soilless culture, which is considered the most efficient nutrient-management system, use of fertilizer can be greatly reduced (Fig. A.4).

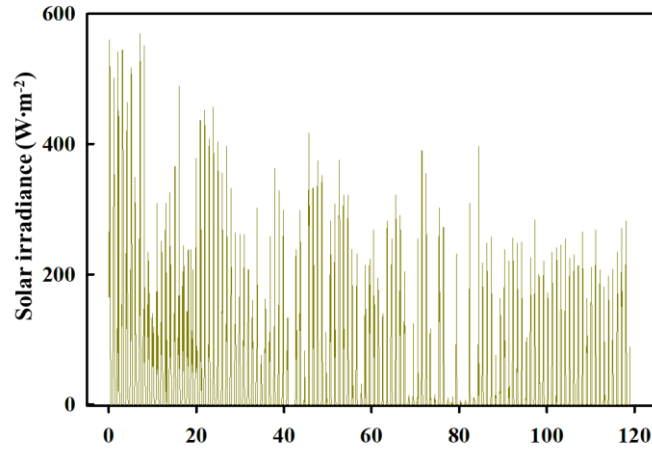


Fig. A. 1. Solar irradiance changes used as input data for simulation of evapotranspiration and irrigation control by a radiation integral method in a closed-loop soilless culture system (Chapter 3).

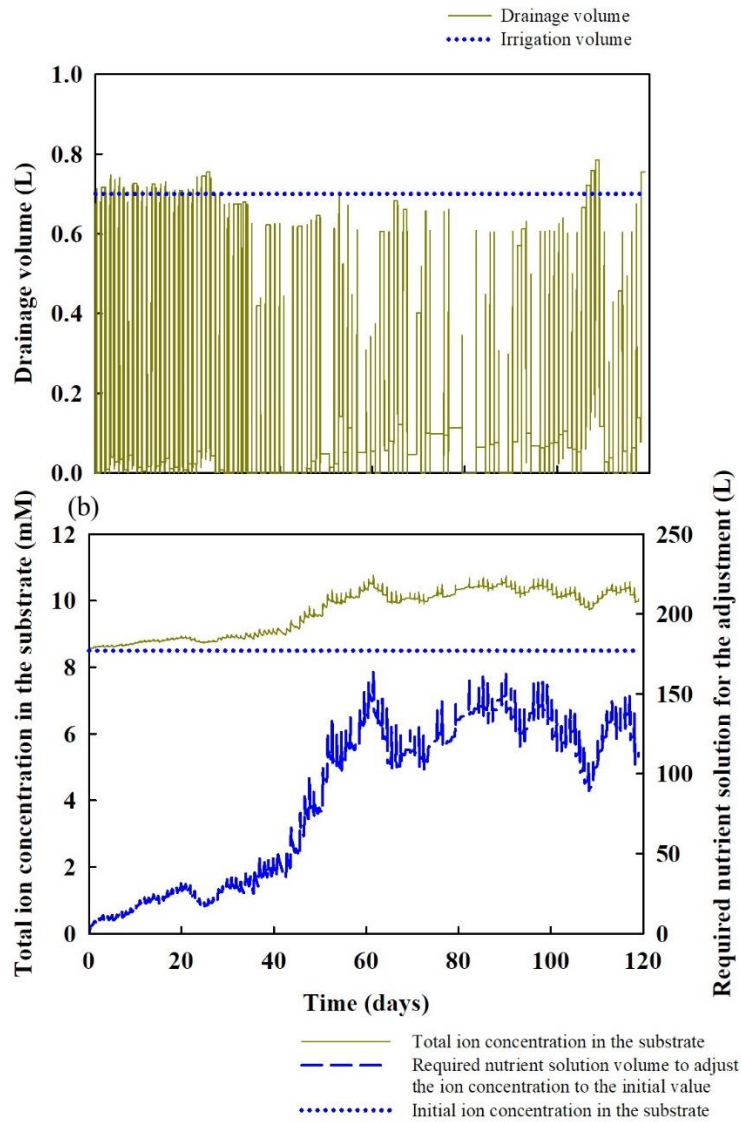


Fig. A.2. Drainage volume changes in the closed-loop soilless culture system using the conventional nutrient-replenishment method (a) and the irrigation volume indicate the supplied nutrient solution per irrigation event. Total ion concentration in the substrate and volume of nutrient solution required for adjustment of ion concentration to the initial value at each time step (b); the conditions used for

calculation of the amount of nutrients required for the adjustment are as follows: initial total concentration (C_i) is 8.5 mM and total ion concentration of nutrient solution for the adjustment (C_d) is 8.4 mM; a dilution equation was used to calculate the volume of required nutrient solution (V_d) for the adjustment: $V_d = (C_c V_c - C_i V_c) / (C_i - C_d)$, where V_c is the current volume of nutrient solution in the substrate, and C_c is the current total ion concentration in the substrate (Chapter 3).

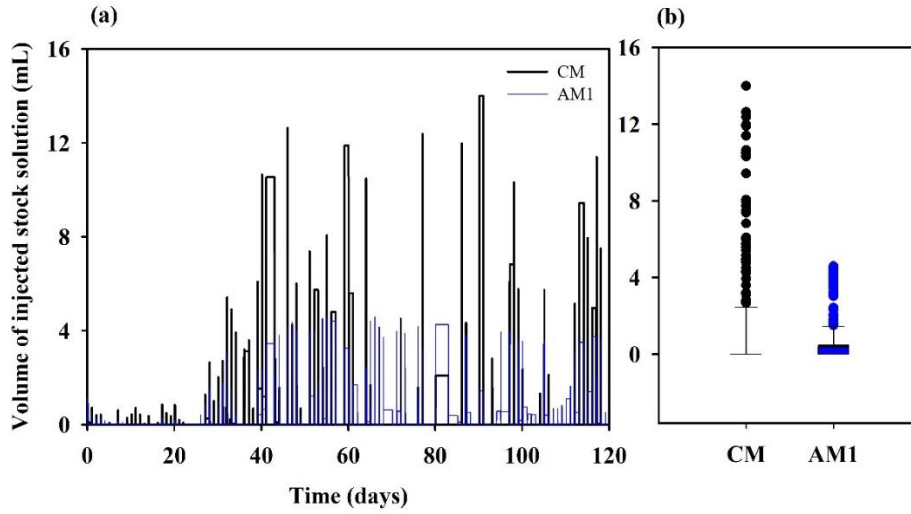


Fig. A.3. Changes in volume of injected stock solution (a) and box-plot comparison (b).

The box plot shows different distributions in the volume of stock solution injection during simulation between the conventional nutrient-replenishment method (CM) and the alternative mixing method with estimated field capacity (F_{est}) using Eq. 3-14 (AM1) (Chapter 3).

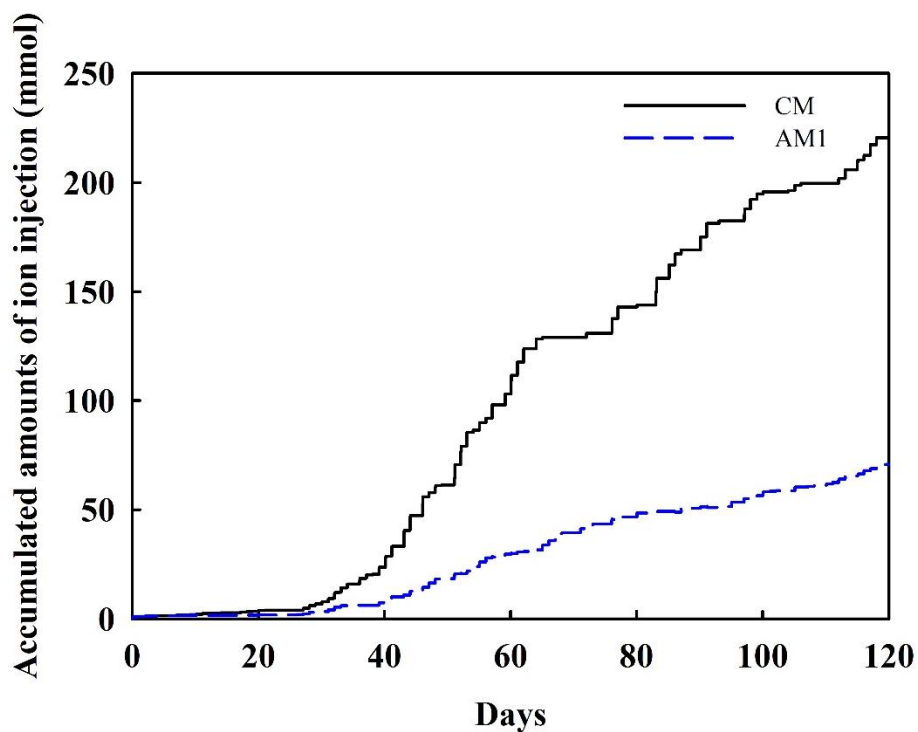


Fig. A.4. Accumulated amount of injected ions in the soilless culture system according to the nutrient-replenishment method: conventional nutrient-replenishment method (CM) and alternative nutrient-replenishment method with estimated field capacity (F_{est}) using Eq. 3-14 (AM1) (Chapter 3).